CONFERENCE PROCEEDINGS

HEALTH SCIENCES

A Comparative Study Among *Thunbergia Laurifolia* Lindl. Extract Cream, 0.02%TriamCinolone Cream and Cream Base for the Treatment of Plaque-Type Psoriasis

Siripan Toemmongkonchai MD[1], Thep Chalermchai[2] MD PhD

[1]school of Anti-Aging and Regenerative Medicine,
[2]School of Anti-Aging and Regenerative Medicine, Mae Fah Luang University, Bangkok, Thailand
**Corresponding author. E-mail: Thep_chalermchai@hotmail.com*

Abstract

Psoriasis is a chronic skin disorder that commonly treated with topical corticosteroids. Recent studies indicated that *Thunbergia laurifolia Lindl.* leaf extract, called "Rang Chuet" in Thai, could reduce inflammation and anti-oxidative effects. An clinical study of *Thunbergia laurifolia Lindl.* leaf extract for psoriasis is limited. This study was a within-body, double-blinded, randomized controlled comparative study among 10% *Thunbergia laurifolia Lindl.* leaf extract, 0.02% triamcinolone cream, and the cream base (control) for the treatment of plaque-type psoriasis. Each lesion area was randomly assigned for treatment with a different type of tested cream. All patients were instructed to apply the creams twice a day for eight weeks and followed every two weeks. Skin improvement evaluations included the mean change of modified psoriasis area severity index (mPASI score) and mean change of their quality of life (DLQI score) and any adverse reactions. Thirty subjects completed the study protocol. Treatments with 0.02% triamcinolone cream and 10% *Thunbergia laurifolia Lindl.* leaf extract cream demonstrated significantly better improvement for mPASI score , proportion of those subjects reached 50% improvement at 8-week visit by mPASI 50 and DLQI score than the cream base group ($p < 0.001$). But 0.02% triamcinolone cream was slightly better reduction of mPASI score than 10% *Thunbergia laurifolia Lindl.* leaf extract cream but did not significant difference ($p = 0.63$). Only 2 patients (6.75) in 0.02% triamcinolone cream achieved a score of mPASI 75 at 8 week visit. There were 4 subjects (11%) for 10% *Thunbergia laurifolia Lindl.* leaf extract cream, developed skin reaction. In conclusion, 10% *Thunbergia laurifolia Lindl.* leaf extract cream is effective and safe for the treatment of plaque type psoriasis .The adverse effect is mild skin reaction. Therefore, use of 10% *Thunbergia laurifolia Lindl.* leaf extract cream can be an alternative treatment for plaque type, psoriasis.

Keywords: *Thunbergia laurifolia Lindl*, Plaque-Type Psoriasis

Introduction

Psoriasis is a chronic skin disorder that directly affects their physical and mental health. Most of them require the lifelong care and specific treatment. Plaque psoriasis is the most common form of psoriasis, occurred in more than 80 % of cases.[1]

Psoriasis is an unknown etiology but postulated that this disease may cause by an abnormality of the immune system resulting in chronic inflammation and hyper-proliferation of keratinocytes which characterize by skin redness and scaling at lesional site.

Various kind of topical corticosteroids in different strength are often used in the treatment of psoriasis, but an adverse effect is common for long term usage including skin atrophy, telangiectasia or or striae of the skin.[2]

Thunbergia laurifolia Lindl. leaf extract cream is a Thai herbal medicine contain many medicinal properties include, reduction of inflammation, vasodilatation and permeability effect, reducing symptoms related to inflammation such as pain, swelling, redness, and burning sensation. It also stimulates the wound healing process and promotes an antioxidation and anti mutagenic effect in chronic inflammatory wound or skin ulceration.

Little is known about clinical efficacy and safety of this Thai herbal medicine, *Thunbergia laurifolia Lindl.* leaf extract for the treatment of psoriasis in previous study elsewhere.

Materials and Methods

Objective

This study aimed to compare the efficacy and safety among *Thunbergia laurifolia Lindl.* extract cream, 0.02% triamcinolone cream and creambase for the treatment of plaque-type psoriasis

Research methodology

This clinical study was approved by Mae Fah Luang University Ethical committee. Thirty-six subjects with mild severity (less than 10% body surface area

involvement) plaque-type psoriasis voluntarily signed in consented form and willing to participant in this study and were enrolled during December 1, 2015 - February 28, 2016. This is intra-individual split area study, by selecting the targeted lesion in 3 different tested sites. For each subject, 3 different lesions will randomly assigned to receive either (1) 10% *Thunbergia laurifolia Lindl.* leaf extract cream or (2) 0.02% triamcinolone cream or (3) cream base.

All of study subjects were instructed to apply the tested creams over the tested area twice a day during the study period for 8 weeks and advised to follow up every 2 weeks for clinical assessments. The clinical evaluation and outcomes included modified psoriasis area severity index (mPASI) score, 50% and 75% improvement at 8-week visit by comparing to the baseline of mPASI score (mPASI 50 and mPASI 75) , their quality of life by Dermatology Life Quality Index (DLQI score) and adverse effects.

Statistical Analysis

Descriptive statistics for demographic data, , the number of skin lesions that achieved mPASI 50 and mPASI 75 scores and adverse effects, plan to summarize as frequency (%) for qualitative data and report with average mean and standard deviation (SD) for quantitative data.

Inferential statistics were used to compare the average mean changes from the baseline of mPASI scores and DLQI scores at 8-week visit.

The proportion of rashes that achieved mPASI 50, and mPASI 75 scores were compared between the treatment methods using the Chi-square test.

Level of statistical significance will set at $p \leq 0.05$.

Results

From the total of 36 subjects, thirty subjects completed the study. Six subjects (16.7%) withdrawal from the study, due to skin reaction to any tested creams. There were 17 males (56.7%) and 13 females (43.3%). Average mean (SD) age was 42.2 (9.4) years (range; 20-59). Average age at the onset of psoriasis was 33.8 (9.3) years. All 30 subjects (100%) had positive history for previously treated with topical steroid treatment prior to the study.

Mean change from the baseline to 8-week visit of mPASI score

Clinical improvement was reported by the gradual significant reduction of mPASI scores for all 3 groups of treatments. Treatments with 0.02% triamcinolone group and 10% *Thunbergia laurifolia Lindl.* leaf extract cream demonstrated significantly better improvement for mPASI score than the cream base group ($p < 0.001$) (Figure 1). Treatment with 0.02% triamcinolone cream was slightly better reduction of mPASI score than 10% *Thunbergia laurifolia Lindl.* leaf extract cream but did not significant difference ($p = 0.63$).

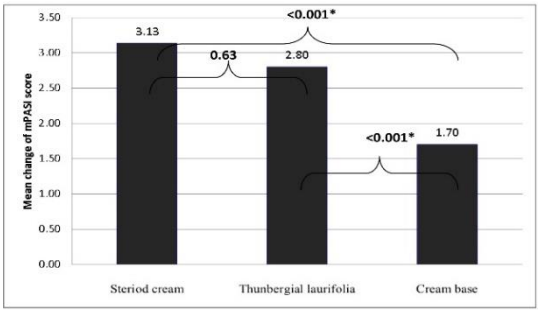


Figure 1: To compare average mean change from the baseline of mPASI score among 0.02% triamcinolone cream, 10% *Thunbergia laurifolia Lindl.* leaf extract cream and creambase.

mPASI 50 and mPASI 75

Treatments with 0.02% triamcinolone cream and 10% *Thunbergia laurifolia Lindl.* leaf extract group had significantly greater proportion of those subjects reached 50% improvement at 8-week visit by mPASI 50 (50% and 43.3%, respectively) than the creambase (3.3%) ($p < 0.001$, Figure 2). There was no difference between 0.02% triamcinolone cream and 10% *Thunbergia laurifolia Lindl.* leaf extract cream group. Only 2 patients (6.75) in 0.02% triamcinolone cream group achieved a score of mPASI 75 at 8 week visit.

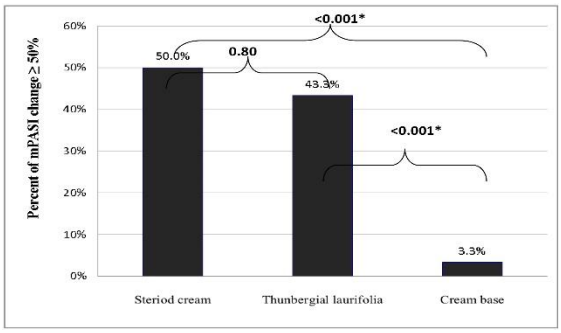


Figure 2: To compare proportion of those subjects reached 50% improvement at 8-week visit by mPASI 50 among the 3 groups

Dermatology Life Quality Index (DLQI score)

The quality of life of all subjects significantly improved over time by the reduction of DLQI score from the baseline to week 8th for all 3 groups. Treatment with 0.02% triamcinolone group demonstrated the highest score reduction (6.9 points), followed by 10% *Thunbergia laurifolia Lindl.* leaf extract group (5.13 points) and significantly better than the base cream with only 1.87 points reduction ($p < 0.001$, Figure 3).

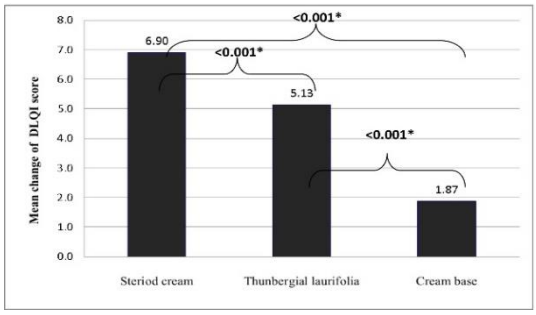
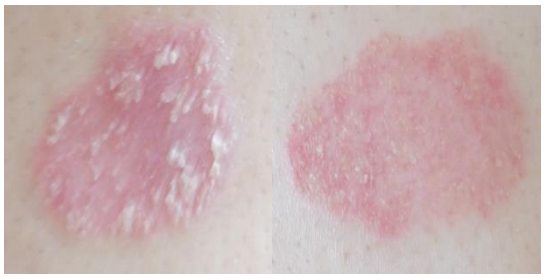


Figure 3: To compare average mean reduction from the baseline of DLQI score among 0.02% triamcinolone cream, 10% *Thunbergia laurifolia Lindl.* leaf extract cream and the cream base.



Before After

Figure 4: Showed clinical improvement by reduction of skin redness and thickness, scaling and mPASI score after 8-week treatment of 10% *Thunbergia laurifolia Lindl.* extract cream

Adverse events:.

There were 11% for 0.02% triamcinolone cream, 11% for 10% *Thunbergia laurifolia Lindl.* leaf extract cream and 2.8 % for cream base developed skin reaction.

Discussion

Currently, a lack of evidence to confirm an efficacy of traditional Thai herbal medicine "Rang Chuet" elsewhere. This study aimed to evaluate a safety and efficacy of *Thunbergia laurifolia Lindl.* leaf extract cream for the treatment of chronic skin disorder, plaque type psoriasis. This study demonstrated that 10% *Thunbergia laurifolia Lindl.* leaf extract cream contain equal efficacies in term of mPASI score and quality of life score (DLQI) as 0.02% triamcinolone cream and significantly better than the cream base group. These anti- inflammatory effect results were consistent with several previous studies indicating that 10% *Thunbergia laurifolia Lindl.* leaf extract could reduced cellular inflammation in mice Boonyarikpunchai W.[3], promoted wound healing in rats Kwansang J.[4] and produced high antioxidant activity in vitro Oonsivilai R.[5]. And also Kwannate B. et al [6] conducted the study of fermented *Thunbergia laurifolia Lindl.* leaf extract for treatment of psoriasis, that showed clinical improvement after treatment of 3 months without clinical recurrence after 9 months.

Conclusion

10% *Thunbergia laurifolia Lindl.* leaf extract cream is effective and safe for the treatment of plaque type psoriasis but had slightly non-significantly inferior to 0.02% triamcinolone cream, but significantly superior to the cream base. Theadverse effect is mild with skin redness and itchy symptoms. Therefore, use of 10% *Thunbergia laurifolia Lindl*. leaf extract cream can be an alternative treatment for plaque type, psoriasis.

Suggestion

A clinical study to elucidate whether a longer period of clinical trial on *Thunbergia laurifolia Lindl.* leaf extract will be will further be required.

Reference

1. Akaraphanth R., Kwangsukstid O., Gritiyarangsan P. and Swanpanyalert N. Psoriasis registry in public health hospital. J Med Assoc Thai. 2013 Aug; 96(8): 960-6.
2. Dermatological society Thailand, clinical practice guideline, 2011.
3. Boonyarikpunchai W., Sukrong S. and Towiwat P. Antinociceptive and anti-inflammatory effects of rosmarinic acid isolated from *Thunbergia laurifolia Lindl.* Pharmacol Biochem Behav. 2014 Sep;124: 67-73.
4. Kwansang J., Itthipanichpong C. and Limpanasithikul W. Evaluation of wound healing activity of *Thunbergia laurifolia* supercritical carbon dioxide extract in rats with second-degree burn wounds. J Adv Pharm Technol Res. 2015 Jul-Sep; 6(3):103-7.
5. Oonsivilai R., Mario G. Ferruzzi and Suwayd Ningsanond. Antioxidant activity and cytotoxicity of Rang Chuet (Thunbergia laurifolia Lindl.) extracts. As. J. Food Ag-Ind. 2008, 1(02): 116-128.
6. Burgbarn K. Adaptative Thai herbal cure psoriasis. Routine to research Thailand.org. 2013.

A Comparative Study of 0.2% Dihydrooxyresveratrol Cream and 2% Alpha Arbutin Cream in Facial Skin Whitenings in Thais

Patthama Paprakhon[1*]

[1]*Master of Science Program in Dermatology, School of Anti-Aging and Regenerative Medicine, Mae Fah Luang University, Bangkok,10110*
[*]*Tel.: +66616645545, E-mail : adioscaro@gmail.com*

Abstract

Dihydrooxyresveratrol, a new derivative of Oxyresveratrol, indicated more potent tyrosinase inhibitory activities compared to Oxyresveratrol. In this study we compared the efficacy of 0.2 % Dihydrooxyresveratrol and 2% Alpha arbutin for facial whitening. Twenty-one Thai volunteers, were enrolled. Study design was Double-blind, randomized, controlled trial. Randomly selected for applied either 0.2% Dihydrooxyresveratrol cream and 2% alpha arbutin cream in a split- face design, twice daily for 12 weeks. Evaluation was performed every 4-week interval using mean melanin index measured by Mexameter MX18, photographs from VISIA® and records of side effects. Volunteers' satisfaction was assessed by questionnaires at 12th week. As a result, both groups demonstrated statistically significant reduction from the baselines at 4th week, 8th week, and 12th week, respectively. And paired differences of mean melanin index showed 0.2% Dihydrooxyresveratrol cream were statistically significant more effective than 2% Alpha arbutin cream in facial whitening at 4th week and 12th week. However, global photographic scores between both sides revealed no statistically significant in results. Most volunteers rated 0.2% Dihydrooxyresveratrol cream as much improvement for satisfactory. Side effects were recognized only mild in both groups. We conclusion that 0.2% Dihydrooxyresveratrol cream were statistically significant more effective than 2% Alpha arbutin cream in facial whitening.

Keywords: Dihydrooxyresveratrol/ Alpha arbutin/ Skin

Introduction

The current value of pale skin in Thais has increased. High cost whitening products were bought monthly for skin maintenance[1]. Most of whitening substances mainly inhibit tyrosinase activity, the key enzyme in melanogenesis [2]. Many companies launched their whitening products that share same mechanism but showed lesser side effects, for examples, Alpha arbutin which has been well-known for its benefits of whitening property and minimal complication[3]. Recently, *Artocarpus Lakoocha* Roxb., which one of Thai herbs that has been generally used as whitening agents[4]. And contains Oxyresveratrol as a major active component.

In vitro The studies revealed that Dihydrooxyresveratrol, a new derivative of Oxyresveratrol from *Artocarpus Lakoocha* Roxb., showed more potent tyrosinase inhibitory activities than Oxyresveratrol for 8 times, and dose-dependent manner[5].

Previous studies of 0.1% Dihydrooxyresveratrol cream showed only small concentration can effectively promote facial skin whitening equally to 2% Alpha arbutin cream[6]. In this research, due to

Dihydrooxyresveratrol that effects to dose-dependent. Accordingly the study had increasing concentration of Dihydrooxyresveratrol to 0.2% to compare efficacy to Alpha arbutin for facial whitening.

Objective

To compare efficacy of 0.2% Dihydrooxyresveratrol and 2% Alpha arbutin for facial whitening in Thais.

Material and Methods

Twenty-one Thai Volunteers (men and women, aged 20-60 years old), with Fitzpatrick Skin type 3-5 were enrolled. The research designed as double-blind, randomized, controlled, split-face clinical trial. 0.2% Dihydrooxyresveratrol cream and 2% Alpha arbutin cream were randomly applied, used block randomization, in a split-face design (right and left sides) twice daily for 12 weeks. Volunteers also received sunscreen. Facial skin whitening was evaluated at 4th, 8th and 12th weeks using mean melanin index measured by mexameter MX18. Photographs from VISIA® Complexion Analysis System before treatment and at

4th, 8th and 12th weeks were compared and scored by 3 dermatologists. Volunteers' side effects were assessed by questionnaires and physician's observation. Volunteers' satisfaction was evaluated at 12th week by questionnaires.

Statistical analysis

Comparison of mean melanin index in the same sides using Paired T-test. Paired differences between both sides were analyzed by Unpaired t-test. Global photographic scores were differentiated by Mann-Whitney U test. Volunteers' satisfaction between both sides and complication were assessed using Fisher-Exact test. The researcher did the following at significance levels of p-value <0.005.

Results

Twenty-one volunteers completed the study. The subjects were female (16/21, 76.2%) and male (5/21, 23.80%). The average participant age was 33.33 ± 8.80 years old. Mean melanin index of the sides that applied 0.2% Dihydrooxyresveratrol cream reduced from baseline before treatment to baseline at 12th week both area (From 269 ± 63.39 to 227.85 ± 62.77 at forehead and from 216 ± 49.20 to 182.28 ± 50.13 at cheeks). Analyzing in group showed 0.2% Dihydrooxyresveratrol cream statistically significant reduction since 4th week (p<0.001). And 2% Alpha arbutin cream reduced from baseline before treatment to baseline at 12th week both area (From 271.47 ± 64.83 to 237.80 ± 67.78 at forehead and from 214.71 ± 48.58 to 188.61 ± 51.36 at cheeks), and significant reduction since 8th week (p<0.001).

Compared differences of mean melanin index of forehead showed 2% Dihydrooxyresveratrol cream were statistically significant more effective than 2% Alpha arbutin cream in facial whitening at 4th week, 8th week and 12th week (p<0.001, p<0.001 and p<0.001).(Figure 1) And paired differences of mean melanin index of cheeks showed 2% Dihydrooxyresveratrol cream were statistically significant more effective than 2% Alpha arbutin cream in facial whitening at 4th week and 12th week (p-value<0.001, p=0.006 and p<0.001).(Figure 2)

However, global photographic scores between 2% Dihydrooxyresveratrol cream was not significantly different to 2% Alpha arbutin cream after 4th week, 8th week and 12th week (p-value=0.870, p=0.571 and p=0.312).(Figure 3) Most volunteers rated 0.2% Dihydrooxyresveratrol cream as much improvement for satisfactory and scored 2% Alpha arbutin cream as moderately satisfied. No statistical difference of volunteers' satisfaction scores (p = 0.57). Observed side effects were only mild in both groups and no statistical significantly difference between both groups (p = 0.58).

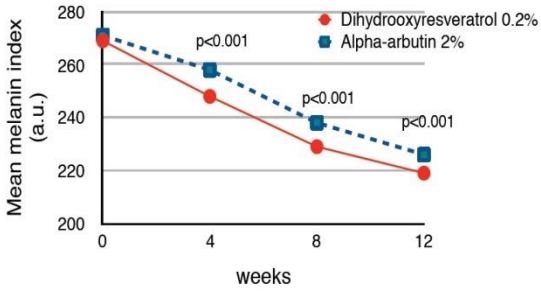


Figure 1 Linear graphs showed comparison of mean melanin index of foreheads that applied 0.2% Dihydrooyresveratrol cream and 2% Alpha arbutin cream in each period.

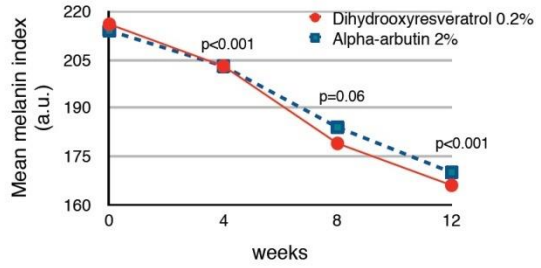


Figure 2 Linear graphs showed comparison of mean melanin index of cheeks that applied 0.2% Dihydrooyresveratrol cream and 2% Alpha arbutin cream in each period.

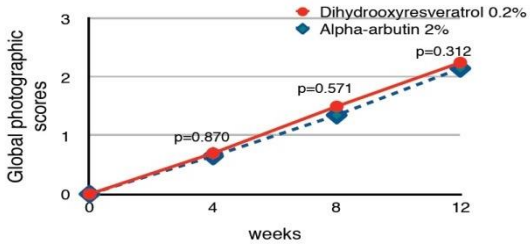


Figure 3 Linear graphs compared differences of mean changes of global photographic scores between two groups**.**

Discussion

From the research, we evaluated that the efficacy of 0.2% Dihydrooxyresveratrol cream were significant more effective than 2% Alpha arbutin cream in both area since 4th week. In comparison to previous study with different concentration of Dihydrooxyresveratrol, 0.1% in prior study showed equally effective [6]. This possible explained by Dihydrooxyresveratrol also inhibited the tyrosinase enzyme in a dose-dependent manner [5]. As a result that increasing the concentration of Dihydrooxyresveratrol cream to 0.2% were more effective. In this study, the melanin content of each application site was measured using Mexameter MX18. When analyzing the reduction of mean melanin index showed 0.2% Dihydrooxyresveratrol cream was more effective agent, giving the faster onset of significant facial whitening effect after applies only 4th week, followed by 2% Alpha

arbutin cream (8th week). The efficacy also increased to maximum observed at 12th week in both groups. This may be explained by the inhibitory efficacy of tyrosinase, which is known to be the key enzyme in melanogenesis. Correlated with *in vitro* study [5]. Dihydrooxyresveratrol showed potent tyrosinase inhibitory activity due to its bibenzyl structure which gave more flexibility and thus allowed the phenolic groups to interact with the tyrosinase enzyme more effectively.

The research outcomes that was evaluated visual evaluation. We used VISIA®, which compared photos in the same positioning and standardized lighting.
Our finding, global photographic scores showed no significantly different between both groups. In contrast to significantly decreasing of mean melanin index. This may be explained by the sensitivity of Mexameter in melanin detection that highly sensitive instrument capable of detecting small changes in the melanin content. So mexameter much greater than visual assessment of skin color change. Volunteers' satisfaction scores rated 0.2% Dihydrooxyresveratrol cream as much improvement. However, no statistical difference of volunteers' satisfaction scores ($p = 0.57$).

A few subjects also reported a minimal dry skin condition in both groups and completely cured without volunteer's discontinuation from the study. This supported previous study reported cytotoxic potential of Dihydrooxyresveratrol that no toxicity in human cells [5]. This condition may be was attributed to the humectant effect of propylene glycol in formula for cream base because it was detected in both two groups of the volunteers.

Conclusion: 0.2% Dihydrooxyresveratrol cream were statistically significant more effective than 2% Alpha arbutin cream in facial whitening.

Conclusion

0.2% Dihydrooxyresveratrol cream is safe and statistically significant more effective than 2% Alpha arbutin cream in facial whitening. And also could be a useful whitening cosmetic product.

Suggestion

We suggest to apply 2% dihydrooxyresveratrol to treatment in other pigmentation disorder, for example, melasma or postinflammatory hyperpigmentation. However, more studies are needed to confirm the clinical efficacy and safety of Dihydrooxyresveratrol in larger number of subjects using different formulations or in combination with other whitening agents.

References

Ruksawong, A. (2011). Behavior of purchasing whitening cream for maintaining face skin of women in Bangkok area. Independent Study of Business Administration. Rajamangala University of Technology Thanyaburi, Pathum Thani.

Gillbro, J. M., & Olsson, M. J. (2011). The melanogenesis and mechanisms of skin-lightening agents - existing and new approaches. International Journal Of Cosmetic Science, 33(3), 210-221.

Petkou, D., Diamantidis G., & Vasilakakis,M. (2002). Arbutin oxidation by pear (*Pyrus communis* L.) peroxidases. Plant Science, 162, 115-119.

Petkou, D., Diamantidis G., & Vasilakakis, M. (2002). Arbutin oxidation by pear (*Pyrus communis* L.) peroxidases. Plant Science, 162, 115-119.

Dej-adisai, S., Meechai, I., Puripattanavong, J., & Kummee, S. (2014). Antityrosinase and antimicrobial activities from Thai medicinal plants. Archives Of Pharmacol Research, (4), 473.

Likhitwitayawuid, K., Sornsute, A., Sritularak, B., &Ploypradith, P. (2006). Chemical transformations of oxyresveratrol (trans-2,4,3',5'-tetrahydroxystilbene) into a tyrosinase inhibitor and a strong cytotoxic agent. *Bioorganic & Medicinal Chemistry Letters, 21*, 5650.

Wiphawee, P.(2014). A comparative study of 0.1% dihydrooxyresveratrol cream and 2% alpha arbutin cream in facial skin whitening in Thais.59-61.

A Comparative Study on the Efficacy of 10% Zinc SulfateSolution And 4% Hydroquinone Topical Treatmentfor the Treatment of Melasma in Thai Nationals.

Pichapuk Jarasrassamee[1] and Paisan Rummaneethron[2*]

[1, 2]*Master of Science Program in Dermatology,School of Anti-aging and Regenerative Medicine, Mae Fah Luang University,Thailand*
[*]*Corresponding author.E-mail:p-om-p@hotmail.com*

Abstract

Melasma, a common disorder of hyperpigmentation, is often difficult to treat. The standard treatment is 4% hydroquinone topical treatment. Although 10% zinc sulfate solution has been reported to be useful for the treatment of patients with Melasma, no clinical trials have been conducted in relation to its safety or efficacy. The aim of this research is to carry out a comparison study as between the efficacy of 10%zinc sulfate solution and that of 4%hydroquinone topical treatment in the treatment of Melasma in Thai nationals.

Twenty two (22) Thai volunteers with bilateral facial malasma were treated with 10% zinc sulfate solution on one side of the face and the other side was treated with 4%hydroquinone topical, this treatment was given twice-daily for 12 weeks with a follow up every 2 weeks.
At the end of every 2 weeks (2,4,6,12), any changes in the disorder was evaluated by means the Maxemeter MX18, MASI score, global satisfactory score and any local side effects.
The twenty two original volunteers fully completed the study.

Both treatments showed a statistically significant reduction from baseline in the mean melanin index and MASI score at the 2, 4 and 6 week evaluations.
The sides of the face treated with 4% hydroquinone had more significant reductions when compared with the mean melanin index at 6 weeks (p=0.023) (4% hydroquinone 296.85+-74.14 to 257.5+-66.46), (10% zinc sulfate 283.33+-85 to 272.39+-66.32) and compared with the MASI score at 2 and 4 weeks (week 2 p=0.041) (4%hydroquinone7.10+-4.17 to 5.5+-3.69) (10% zinc sulfate 6.60+-4.37 to 5.74+-4.28), (week 4 p=0.011) (4% hydroquinone 7.10+-4.17 to 3.86+-2.70) (10% zinc sulfate 6.6+-4.37 to 4.69+-3.78).

There was found to be no statistically significant reduction from baseline at 12 weeks (follow up) (mean melanin index p=0.187 4% hydroquinone 7.10+-4.17 to 3.59+-3.25, (MASI score p=0.183 10% zinc sulfate 6.60+-4.37 to 4.42+-3.83).
The most common side effect of treatment by both 4% hydroquinone and 10% zinc sulfate was a burning sensation.These were well tolerated and no volunteer discontinued participation in the study because of adverse side effects.

The conclusion drawn from the research is that 10% zinc sulfate is not as effective in treating melasma as 4% hydroquinone.
It is suggested that further studies and safety and efficacy trials be carried out to ascertain if by adding zinc to current topical treatments of melasma this could improve the existing treatment response.

***Keywords*:**Hydroquinone, melasma, zinc sulphate

Introduction

Melasma is one of the most common and disturbing cosmetic disorders with skin darkening commonly affecting sun-exposed areas of the body, typically, the forehead, malar,and mandibular regions of the face(1).

It presents as symmetric, hyperpigmented patches with irregular demarcated borders on the face(1, 2).
Melasma is more common in women.
Other known risk factors are pregnancy and some medications such as hormone and antiepileptic drug medication (3).
It is more common in the Asian population than in other races (4). Current treatments have not been successful at completely correcting the skin pigmentation of melasma (5).

Standard treatment include sun avoidance and sunscreen, hypopigment agents, chemical peels,lasers and dermabrasion. (1, 2)
Hypopigmenting agent not only act as tyrosinase inhibitors,but also scatter the melanin throughout the skin.(2)

Hydroquinone, one of the most renowned topical treatments, displays its action through both the inhibitor of tyrosinase and exclusive detriment to melanosomes and melanocytes. 4% hydroquinone is the gold standard for melasma treatment(6,7). Although highly effective in the treatment of melasma,some adverse effects due to hydroquinone therapy include irritant and allergic contact dermatitis, post inflammatory hyperpigmentation, hypopigmentation of the surrounding skin,(6,7) Ochronosis is a rare side effect seen with prolonged use of concentration>2% (8)
Zinc is a main trace element and important nutrient.The effect of zinc acquired through its action includes anti-inflammatory, anti-oxidant, cytotoxic, and healing agent.

Zinc sulfate was found to be effective in the treatment of many dermatological disorders, such as cutaneous leishmaniasis, recurrent herpes simplex, common and genital warts and recently melasma (9,10). According to the evidence, zinc has an important role in skin health, topical zinc has various beneficial effects in the treatment of skin disorders, but there is not enough data establishing that zinc is an effective and beneficial treatment for melasma.

The aim of our study was to compare the efficacy of treatment for melasma between 10% zinc sulfate solution and 4% hydroquinone topical, the latter being the standard treatment for melasma.

Objective

To compare the comparative efficacy of 10% zinc sulfate solution and 4% hydroquinone topical in the treatment of Thai nationals with Melasma.

Materials and Methods

Twenty two Thai volunteers with bilateral facial malasma were treated with 10% zinc sulfate solution to one side of their face and the other side was treated with 4% hydroquinone twice-daily for a period of 12 weeks with follow up every 2 weeks.

After 2, 4, 6, 12 weeks, the treatment was evaluated by Maxemeter MX18, MASI score, global satisfactory score and any local side effects.

Statistic for data analysis

Efficacy analysis

- Evaluation of detailed digital camera photographs taken at 2, 4, 6, 12 weeks comparing the condition of the Melasma at the start of the study and the condition at the above intervals, after the treatment by 10% zinc sulfate and 4% hydroquinone, using chi- square test or fisher exact test

-Evaluation by comparing mean melanin index from Mexameter MX18 before and after treatment by 10% zinc sulfate and 4% hydroquinone, using pair T-test

- Evaluation by comparing mean melanin index from Mexameter MX18 between treatment by 10% zinc sulfate and 4% hydroquinone, using student T-test

-Evaluation side effects in the treatment by 10% zinc sulfate and 4% hydroquinone,using chi-square test

-Evaluation by comparing the satisfaction between the treatment by 10% zinc sulfate and 4% hydroquinone, using Fisher exact test

The confidence level is specified at 95%. Significant levels for all analyses were set at p-value<0.05

Results

The original twenty two volunteers completed the study. Both sides of the face showed a statistically significant reduction from baseline in mean melanin index and MASI scores. The sides of the face treated with 4% hydroquinone had a more significant reduction when compared with mean melanin index a t 6 weeks(p=0.023) (4% hydroquinone 296.85+-74.14 to257.5+-66.46 ,(10%zinc sulfate 283.33+-85 to 272.39+-66.32) compared with the MASI score at 2, 4 weeks (week 2 p=0.041) (4%hydroquinone7.10+-4.17 to 5.5+-3.69) (10% zinc sulfate 6.60+-4.37 to 5.74+-4.28), (week 4 p=0.011) (4% hydroquinone 7.10+-4.17 to 3.86+-2.70) (10% zinc sulfate 6.6+-4.37 to 4.69+-3.78).

There was no statistically significant reduction from baseline at 12 weeks (follow up)(mean melanin index (p=0.187) (4% hydroquinone 7.10+-4.17 to 3.59+-3.25), MASI score (p=0.183) (10% zinc sulfate 6.60+-4.37 to 4.42+-3.83).

The most common side effect of treatment by 4% hydroquinone and 10% zinc sulfate was that of a burning sensation in the treated areas.These were well tolerated and no volunteer discontinued participation in the study because of adverse side effects.

For the patients satisfaction score, treatment by 4% hydroquinone was significant when compared to treatment by 10% zinc sulfate at 12 weeks (p=0.023)

For the doctors satisfaction score, treatment by 4% hydroquinone was significant when compared to treatment by 10% zinc sulfate at 6,12 weeks (p=0.001,p=0.010)

axis y=difference of melanin index

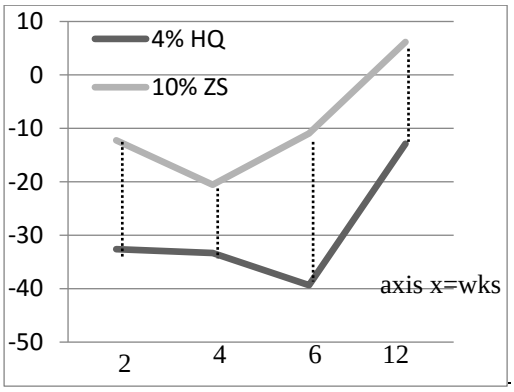


Figure 1. Difference of mean melanin index week 2, 4, 6, 12 in treatment of melasma between 4% hydroquinone and 10% zinc sulfate

Axis y=difference of MASI

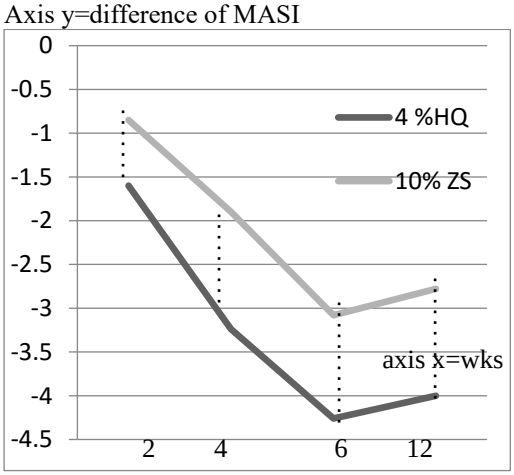
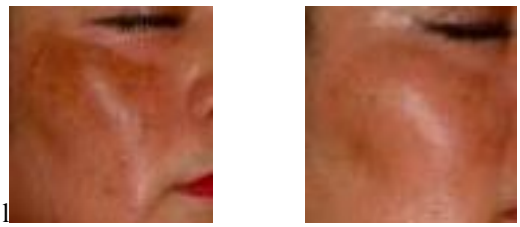
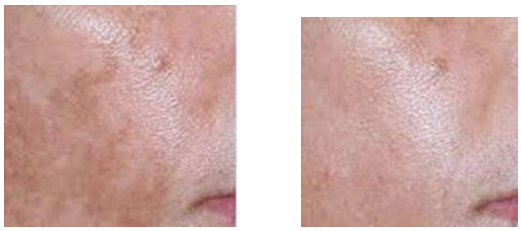


Figure 2. difference of MASI score at 2, 4, 6, 12 weeks in treatment of melasma between 4% hydroquinone and 10% zinc sulfate.



Left Right

Picture 1. Volunteer applied 4% hydroquinone topical. Left is taken at week 1, before treatment. Right is taken at 12 weeks after treatment.



Left Right

Picture 2. Volunteer applied 10% zinc sulfate solution. Left is taken at week 1, before treatment.
Right is taken at 12 weeks after treatment.

Discussion

Malasma is a skin hyperpigmentation disorder that does not resolve completely in most patients with current medications (1, 2 ,5).

For the first controlled comparative trial, we considered zinc as a possible treatment for melasma because of its role in skin health. Zinc has antioxidants and anti inflammatory properties, which may act as regenerative substance in damaged skin. It can also protect skin, and so may prevent extra activity of melanocytes (11, 12). These mechanisms make zinc a potential therapeutic option for the treatment for melasma.

Our data showed that topical zinc therapy is not highly effective for melasma. Although it significantly reduced the MASI score and mean melanin index the MASI score and mean melanin index fell more in 4% hydroquinone.

In the study by Sharquire and colleagues, treatment with topical 10% zinc sulfate for 2 months resulted in 49.8%improvement in MASI score(from 9.45 to 4.70)(10).This greater percentage improvement in MASI score compared with our data may be the result of several factors.

In our study, using the MASI score indicates a reduction in both treatments after 6 weeks. However, this reduction was greater in 4% hydroquinone than 10% zinc sulphate (4.26+-2.83 in 4% hydroquinone, 3.08+-2.54 in 10% zinc sulphate).The baseline of MASI score in 4% hydroquinone and 10% zinc sulfate were7.10+-4.17 and 6.60+-4.37 respectively.
Leenutaphong et al. showed a reduction of 60% in MASI score in patients receiving only sunscreen(13)

Although the previous study of Sharqui regarding treatment of melasma by topical zinc sulphate is informative it demonstrated that the beneficial effect of topical zinc sulphate treatment is only attributable to the sunscreen effect and was not quantitatively significant.

Summary

10% zinc sulfate is not as effective in treating melasma as 4% hydroquinone

Suggestion

It is possible that adding zinc to current topical treatments of melasma could increase the treatment response, but further trials regarding the safety and efficacy of this possibility would need to be performed prior to such a change being implemented.

References

1. Pawaskar MD, Parikh P, Markowski T, McMichael AJ, Feldman SR, Balkrish R. *Melasma and its impact on health-related Quality of life in Hispanic woman.J Dermatolog Treat.2007;18:5-9*.
2. Grimes PE,Melasma: *Etiologic and the therapeutic considerations.Arch Dermatol.1995;131:1453-7.*
3. Sheth VN, Pandya AG, *Melasma:a comprehensive update:part I.J Am Acad Dermatol 2011;65:689-97*
4. Les CS, Lim HW. *Cutaneous disease in Asians,Dermatol Clin 2003;21:669-77*
5. Rajaratnam R,Halpern J,Salim A,Emmett C, *Intervention for melasma.Cochrane Database Syst Rev 2010;7:CD003583.*
6. Rendon M, Berneburg M, Arellano I, Picardo M. *Treatment of melasma.J Am Acad Dematol.2006;54(Suppl):S272-81.*
7. Ennes SBP, Paschoalick RC. Mota de A velar Alchorne M.A *Double-blind,comparative, placebo-controlled study of the efficacy and tolerability of 4%hydroquinone as a depigmenting agent in melasma.J Dermatolog Treat.2000;11:173-9*
8. Palumbo A, d'ischia M, Misuraca G, Prota G. *Mechanism of inhibition of melanogenesis by hydroquinone.Biochim Biophys Acta.1991;1073:85-90*
9. Shrquie KE, Khoesheed AA, Al-nuaimy AA. *Topical zinc sulphate solution for treatment of viral wart.Saudi Med J.2007;28:1418-21*
10. Shrquie KE, Al-Mashhadani SA, Salman HA. *Topical 10% zinc sulfate solution for treatment melasma. Dermatol Surg.2008;34:1346-9*
11. Rostan EF, DeBuys HV, Madey DL, Pinnell SR. *Evidence supporting zinc as an importing antioxidant for skin.Int J Dermatol 2002;41:606-11*
12. Prasad AS. *Clinical manifestation of zinc deficiency.Annu Rev Nutr 1985;5:341-63*
13. Leenutapong V, Nettakul A, Rattanasuwon P. *Topical isotretinoin for malasma in Thai patients a vehicle controlled clinical trial.J Med Assoc Thai.1999;82:867-75*

A Comparative Study of the Efficacy and Satisfactory Evaluation between Burmese Traditional Topical Cosmetic Cream; "Thanaka" and Standard Cream Base for the Treatment of Melesma in Thai People

Su Myat Thinzar[1], Sunisa Thaichinda[2]*

[1] *School of Anti-Aging and Regenerative Medicine, Mae Fah Luang University, 38/11-13 Asoke Place Building, Asoke Road, Sukhumvit 21, Klong Toey Nua, Wattana, Bangkok, 10110, Thailand*

[2] *School of Anti-Aging and Regenerative Medicine, Mae Fah Luang University, 333 Moo1, Thasud, Muang, Chiang Rai 57100 Thailand*

** Corresponding author. E-mail: dr.sunisa.t@gmail.com, yurikosue@gmail.com*

Abstract

Melasma is one of the most common pigmentary skin disorders. The diagnosis of melasma is essentially clinical, and its management is challenging because it is a chronic condition with common recurrences and is often difficult to treat provoking significant emotional and psychological effects in affected patients. *Hesperethusa crenulata* or 'Thanaka' stem bark extract cream which is essential skin care regiment for Myanmar women possesses strong anti-inflammatory, significant anti-oxidation, mild tyrosinase inhibition and slight anti-bacterial activities. However, no clinical study demonstrating the efficacy of 60% Thanaka bark extract cream in the treatment of melasma for Thai people. The Objective is to study the efficacy and satisfactory evaluation of topical herbal cream Thanaka for the treatment of melasma in Thai people. Thirty female with Fitzpatrick skin type III–VI with melasma were enrolled. 60% Thanaka bark extract cream and standard cream base were comparatively applied, in split face design twice daily for 12 weeks. MASI scoring and Mexameter measurement were performed along with photography by VISIA Analysis at baseline, 4th, 8th and 12th week. Adverse events and satisfaction score were observed and recorded throughout the study. Thirty female (aged 30- 65 years, mean age 43±8.32) completed the studies. Mean MASI scores before the treatment for Thanaka and standard cream base are 16.10±4.51 and 16.17±4.64 respectively while Thanaka scoring dropped to 10.48±4.12 and cream base to 12.85±4.364 after the treatment. Its p-value (0.033) was statistically significant. Furthermore, mean melanin index of the sides that applied Thanaka cream (From 263.02±54.30 to 217.99±47.55) and standard cream base (From 267.57±57.72 to 257.46±60.15) demonstrated statistically significant reduced (p value = 0.002) from baselines to final week correspondingly. Most volunteers rated Thanaka cream as much improvement for satisfactory results without serious adverse effects. Twelve weeks of clinical data and studies have allowed for optimization of treatment parameters with improved patient outcomes. 60% Thanaka bark extract cream could have significant better outcomes in all aspects compared with standard cream base from 8th week onwards. Patients were also significantly satisfied with Thanaka cream. Thus, Thanaka is effective to treat melasma without serious side effect.

***Keywords*:** Melasma/Thanaka/efficacy/satisfactory evaluation

Introduction

Melasma is a human melanogenesis dysfunction that results in localized, chronic acquired hypermelanosis of the skin[1]. Risk factors have been associated with among women with Dark-haired persons (Fitzpatrick skin types III–VI) are more susceptible to melasma and also topical cosmetics and fragrances as well as phototoxic drugs can also trigger or aggravate melasma [2].

The diagnosis of melasma is essentially clinical, and its management is challenging because it is a chronic condition with common recurrences and is often difficult to treat provoking significant emotional and psychological effects in affected patients [3].

First-line therapy usually consists of topical compounds that affect the melanin synthesis pathway, broad spectrum photo protection, and camouflage. A thorough understanding of the risks and benefits of various therapeutic options is crucial in selecting the best treatment [2].

Burmese traditional medicine has been in use for centuries. However, in contrast to Chinese traditional medicine, Burmese traditional medicine body of knowledge has not been as well-organized and is in urgent need of scientific management and organization[4].

The stem bark powder of *Hesperethusa crenulata* or 'Thanaka' has been used on the face by Myanmar women for more than two thousand years as a skin care regiment [5]. Thanaka is 100% organic and natural. It's

a yellowish-white cosmetic creamy paste .To make the organic thanaka paste cosmetic, the wood and bark is finely ground and mixed with water. The liquid paste produced has an aromatic fragrance and is the favorite natural skin beautifier in Myanmar for ladies from all walks of life.

According to the previous study, it had been concluded that the water extract of this Thanaka bark possesses significant anti-oxidation and anti-inflammatory activities and mild tyrosinase inhibition activity [5]. Therefore, our study is to conduct that mild tyrosinase activity of Thanaka would actually be useful in inhibition of melanin production. Hence, the main purpose is to study the efficacy and satisfactory evaluation of Burmese traditional topical herbal cream Thanaka on the treatment of melasma in Thai people [5].

Materials and Methods

Thirty female with Fitzpatrick skin type III–VI with melasma were enrolled.

From the formula[6],

Where α = 0.05(two-tailed) , $Z_{0.025}$ = 1.96

β = 0.10 ,$Z_{0.100}$ = 1.28

$$n = \frac{(Z_{\alpha/2} + Z_{\beta})^2 (\sigma_1^2 + \sigma_2^2)}{\mu_d^2}$$

Note

n = sample size per group

σ = standard deviation,

μ_d = Mean difference

60% Thanaka bark extract cream and Standard cream base were comparatively applied, in split face design (Thanaka on right and standard cream base on left sides respectively) twice daily for 12 weeks. MASI scoring and Mexameter measurement were performed along with photography by VISIA Analysis at baseline, 4th week, 8th week and 12th week. Adverse events and satisfaction score were observed and recorded throughout the study.

Statistical Analysis

General demographic data of volunteers used descriptive statistical analysis to provide descriptive information, such as percentages, means, modes, medians, ranges, standard deviations. MASI score and mean melanin index scoring between Thanaka and Standard cream base was analyzed through ANOVA test. Fisher's exact test was used for doctor satisfaction by 3 dermatologists for finding p value on 4th, 8th, 12th week respectively. Patient satisfactory score on 12th week by chi square test n side effects were also noted, the researcher did the following at significance levels of *p-value* <0.05.

Results

Table 1. Demographic Data

DEMOGRAPHIC	n	%
Gender		
Female	30	100.00
Age		
Mean+/- SD (years)	43.00±8.321	
Min- Max(years)	30-58	
Occupations		
1.Goverment officer	8	26.7
2.Business owner	9	30.0
3.House wife	8	26.7
4.Student	0	0
5.employee	3	10.0
6.others	2	6.7
Underlying Disease		
YES	4	13.3
NO	26	86.7
Photosensitivity or Drug-induced hypersensitivity?		
YES	0	0
NO	30	100
Personal medication		
YES	0	0
NO	30	100

Table 1. Demographic Data (continued)

History of food or drug allergy?		
YES	2	6.7
NO	28	93.3
History of following treatment Before this study?		
NO	23	76.6
Intensed Pulsed Light(IPL)	2	6.7
Chemical Peeling	3	10.0
Facial whitening Treatment	2	6.7
Average time exposure to the sunlight (10 am to 4pm)?		
YES	17	56.7
NO	13	43.3
Exposure to sunlight duration?		
Mean+/-SD (min.)	16.33±18.75	
Min-Max (min)	0-60	
Fitzpatrick skin type		
Type III	16	53.3
Type IV	10	33.3
Type V	4	13.4

Table 1 demonstrated the demographic data of the subjects. The mean age of the subject was 43.00±8.321 years, and there were 9 business owners, 8 Government Officers, 8 housewives, 3 employees and 2 people working for other occupations. Furthermore, some of the volunteers have history of using facial whitening

treatment before this study such as 2 subjects who used IPL, 3 chemical peeling. Majority of the subjects had Fitzpatrick skin type III (16/30) the second common one was type IV (10/30) while the rest is type V (4/30). More than half of the subjects (17/30) got exposed to UV light during 10am to 4pm and all the subjects were free from using personal medication but 2 of the subjects had allergy for seafood.

As shown in figure 1, mean MASI scores before the treatment for Thanaka and standard cream base are 16.10±4.51 and 16.17±4.64 respectively while Thanaka scoring dropped to 10.48±4.12 and cream base to 12.85±4.364 after the treatment. Its p-value (0.033) was statistically significant as well.

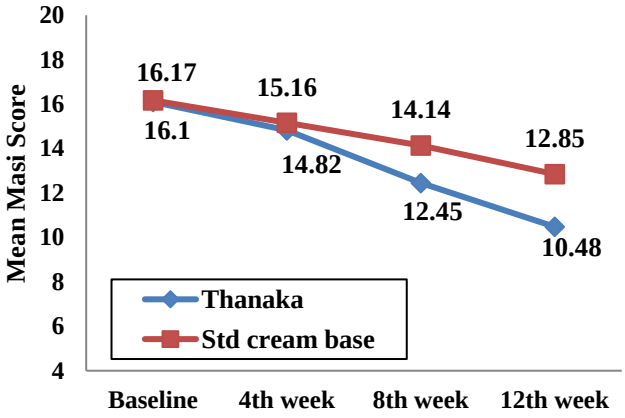


Figure 1 Linear graph showed comparison of mean MASI score in each visit between sides performed Thanaka and Standard cream base

Furthermore Mean melanin index of the sides that applied 60% Thanaka bark extract cream (From 263.02±54.30 to217.99±47.55) and standard cream base (From 267.57±57.72 to 257.46±60.15) demonstrated statistically significant reduced (p value = 0.002) from the baselines to 12th final week correspondingly(figure 2).

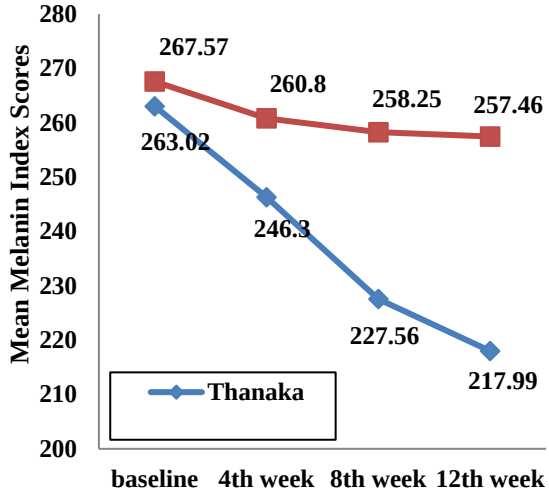


Figure 2 Linear graph showed comparison of mean melanin index score in each visit between sides performed Thanaka and Standard cream base

However, according to figure 3, 4 and 5 doctor evaluation of satisfactory score throughout 12 week revealed no statistically significant in results. Most volunteers rated Thanaka cream as much improvement for satisfactory without serious adverse effects (figure6).

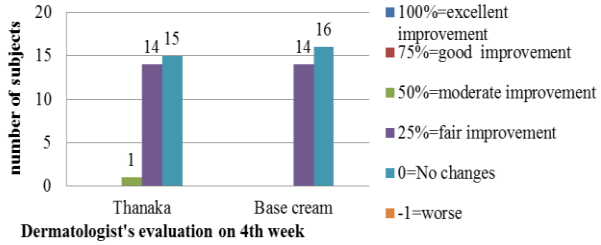


Figure 3 Column chart reveals the frequencies of the dermatologist's evaluation scoring of Thanaka and Standard cream base on 4th week

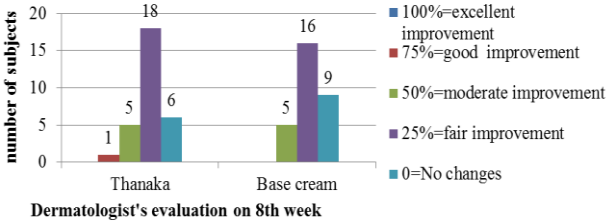


Figure 4 Column chart reveals the frequencies of the dermatologist's evaluation scoring of Thanaka and Standard cream base on 8th week

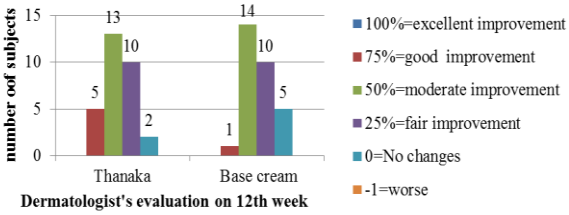


Figure 5 Column chart reveals the frequencies of the dermatologist's evaluation scoring of Thanaka and Standard cream base on 12th week

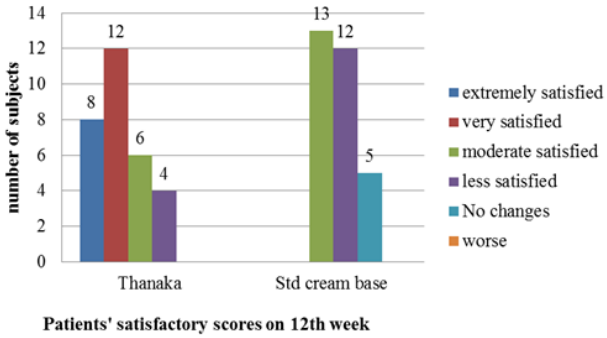


Figure 6 Column chart reveals the frequencies of patients' evaluation scoring of Thanaka and Standard cream base on 12th week

Figure 7 before and after 12 week pictures of one of the volunteers that applied 60% Thanaka bark extract cream

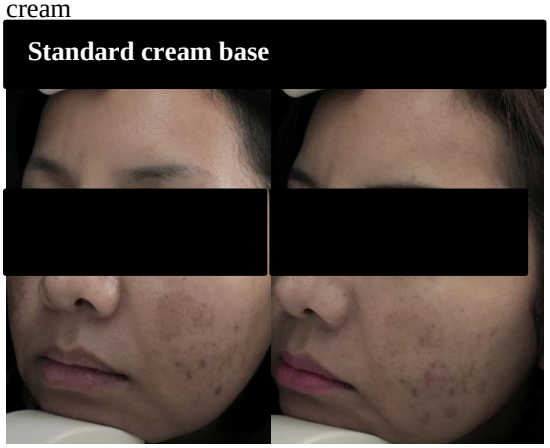


Figure 8 before and after 12 week pictures of one of the volunteers that applied Standard cream base

Discussion

According to this study, all the volunteers were Thai female with Fitzpatrick skin type III–VI respectively. It is possible that women are concerned about their cosmetic and social implications than men. In this study, average age was 43.00±8.321. Main aggravating factors of melanin production were sunlight and average time exposure to sun between 10am-4pm was 16.33±18.75 min. When analyzing the reduction of MASI score between Thanaka and standard cream base for 4th, 8th, 12th week, statistical significance were found on final 12th week prominently. As for mean melanin index between two creams, after 8th week toward final week significantly was reduced. The decreasing of mean melanin index from 60% Thanaka bark extract cream may result from the anti-tyrosinase properties; inhibitor to the key enzyme in melanogenesis and also possesses significant antioxidant and anti-inflammatory activities. Thanaka contains two active ingredients, coumarin and marmesin. Coumarin accounts for the anti-bacterial, anti-fungal, phytochemical, anti-aging and anti-oxidant properties. Wangthong and colleagues performed a series of studies on Thanaka bark [5]. Thanaka antioxidant activity was used as a proxy for anti-aging capacity. Thanaka was found to have mild anti-tyrosinase activity. In an assessment of antibacterial activity, Thanaka showed only slight antibiotic activity against both Gram-negative *Escherichia coli* and Gram positive *Staphyloccocus aureus.* This correlated with the studies of [7, 8]. Even though there was no statistically significant for 3 dermatologists' evaluation, patient satisfactory score on 12th week was significantly satisfied without side effect. Treatment with thanaka showed no significant side effects and was well tolerated; therefore, it could be used for longer periods, as part of the initial hyperpigmentation treatment and as maintenance drug. However, further trials are required to assess the combination of this topical drug with others agents and assess its additive effects in the treatment of melasma.

The main limitations of this study were;

1. Due to small sample size, this may have problem with study interpretation
2. The long term followed up cannot be achieved because the time is limited.

However, pigmentation could potentially be worsened during the course of time due to melasma's nature of chronic process. Other than that, other factors likely to contribute to the differences in clinical outcome include the geographic conditions of Thailand i.e. long duration of summer season and excessive UV light exposure[9]. Finally, at the end of final week of this study, significant differences were found between the two creams and Patients were also significantly satisfied with Thanaka cream and they were all gladly would recommend Thanaka cream to their friends and family.

Conclusion

Twelve weeks of clinical data and studies have allowed for optimization of treatment parameters with improved patient outcomes. 60% Thanaka bark extract cream could have significant better outcomes in all aspects compared with standard cream base from 8th week onwards. Patients were also significantly satisfied with Thanaka cream. Therefore, thanaka is effective to treat melasma without serious side effect.

Suggestion

We suggest further investigations to elucidate whether a longer period of study on thanaka effect after off-treatment, comparison of thanaka with gold

standard medicine or even the pathological tissue correlation

Acknowledgement

Firstly, I would like to express my special thanks of gratitude to my professor Sunisa Thaichinda as well as our chairperson professor Thamthiwat Nararatwanchai who gave me the golden opportunity to do this wonderful project on this research article which also helped me in doing a lot of research and I came to know about so many new things I am really thankful to them.

I owe a debt of gratitude to Dr. Thep Chalermchai who guided through the trouble of doing statistically analysis. Furthermore, I would also like to thank my beloved mother and friends who helped me a lot in finalizing this project within the limited time frame. Last but not the least; I take this opportunity to acknowledge the services of total team of publisher and everyone who collaborated in producing this work.

References

1. Handel, A.C., L.D. Miot, and H.A. Miot, *Melasma: a clinical and epidemiological review.* An Bras Dermatol, 2014. **89**(5): p. 771-82.
2. Shankar, K., et al., *Evidence-based treatment for melasma: expert opinion and a review.* Dermatol Ther (Heidelb), 2014. **4**(2): p. 165-86.
3. Ikino, J.K., et al., *Melasma and assessment of the quality of life in Brazilian women.* An Bras Dermatol, 2015.**90**(2): p. 196-200.
4. Nopparatkiat, P., B.N. Nagara, and C. Chansa-ngavej, *Expert system knowledge acquisition for melasma skin diagnosis and treatment with Thai herbal medicine.* Int. Journal of Applied Sciences and Engineering Research, 2012. **1** (5).
5. Wangthong, S., et al., *Biological activities and safety of Thanaka (Hesperethusa crenulata) stem bark.* JEthnopharmacol, 2010. **132**(2): p. 466-72.
6. Dattalo, P., *Determining SampleSize:Balancing Power, Precision, and Practicality*, ed. T. Tripodi and O.S.U. Professor Emeritus. 2008 New York: Oxford University Press
7. Goldsberry, A., A. Dinner, and C.W. Hanke, *Thanaka: traditional Burmese sun protection.* J Drugs Dermatol, 2014. **13**(3): p. 306-7.
8. Nayar, M.N.S., C.V. Sutar, and M.K. Bhan, *Alkaloids of the stem bark of Hesperethusa crenulata.* Phytochemistry, 1971(10).
9. Li, Y.H., et al., *Efficacy and safety of intense pulsed light in treatment of melasma in Chinese atients.* Dermatol Surg, 2008. **34**(5): p. 693-700; discussion 700-1.

A Comparative Study on the Efficacy of Non-Thermal Plasma Device Combined with Topical Treatment Versus Topical Treatment Alone for Moderate Facial Acne Vulgaris in Thais

Chayanid Bunsaisup[1] and Paisal Rummaneethorn[2*]

[1]*Student of Master of Science Program in Dermatology, School of Anti-Aging and Regenerative Medicine, Mae Fah Luang University Hospital, 38/11-13 Asoke Place Buliding, Asoke road, Sukhumvit 21, Klong Toey Nua, Wattana, Bangkok 10110*
[2]*Advisor, Dean of School of Anti-Aging and Regenerative Medicine, Mae Fah Luang University Hospital, 38/11-13 Asoke Place Buliding, Asoke road, Sukhumvit 21, Klong Toey Nua, Wattana, Bangkok 10110*
[*]*Corresponding author. Email: paisalrr@yahoo.com*

Abstract

Acne is common problem in dermatologic clinic**.** Most of the cases were found in teenagers**.** In many cases showed that Thai conventional treatment may not be effective therapy. There are many alternative treatments which are new technology and reported to be safe, for instance non-thermal plasma. Plasma medicine has many researches showing their efficiency and safety in treatment of acne with minimal side effect
Objective: To evaluate the efficacy of plasma technology in enhancing treatment of acne vulgaris (in term of duration) compared with the topical treatment of acne.
Material and methods: Thirty Thai volunteers were enrolled and had moderate facial acne vulgaris at both sides of face. Randomly assigned for treatment by non-thermal plasma device combined with topical treatment and topical treatment alone in each sides. All volunteers received topical treatment for applying full-face. Half of face would receive non-thermal plasma once a week for 4 weeks. Clinical evaluation was done at week 4, 8, 12 by using photographs of VISIA complexion analysis, acne grading and acne lesion counting. Side effects and satisfaction were assessed by questionnaire.
Results: Twenty eight volunteers completed the study. Both sides of face had statistically significant decrease in acne grade and acne lesion count from baseline. The side of treatment by non-thermal plasma combined with topical treatment had better outcome than topical treatment alone in term of duration, there was statistically significant difference at week 4, 8, 12 when evaluated by comparing acne grade and acne lesion count. The adverse effects resemble between two groups. The satisfaction score of both groups was average to excellent satisfaction.
Conclusion: Non-thermal plasma device combined with topical treatment could enhance the therapeutic efficacy in treatment of moderate facial acne vulgaris in Thais.

***Keywords*:** Non-Thermal Plasma/Acne Vulgaris/Topical Treatment

Introduction

Acne is common problem in dermatologic clinic that can be found in every stage of life for both male and female. Most of the cases were found in teenagers. Unfortunately, Thai conventional treatment may not be effective therapy, however nowadays, there are many alternative treatments of acne vulgaris which are new technology and reported to be safe, for instance non-thermal plasma whose mechanisms of action consist of decreasing sebum production, eradicating *Propionibacterium acnes* (*P. acnes*) bacterium and subside inflammation. These adjunctive therapies have been developed to be more effective and have long-term treatment effects. Many researches showed their efficiency and safety in treatment of acne with minimal side effect, for example some redness or dryness on area of treatment.

Pathogenesis of acne is related to *P. acne* colonization, sebum production, hormonal change and keratin adhesion which blocks sebaceous glands. The main mechanism of action of non-thermal plasma system (NTP) is to generate dielectric barrier discharge (DBD). Benefits of DBD are P. acne sterilization, deceasing inflammation, sebum production and removing keratin plugs and the dead keratin layer. Many previous studies discover that NTP can induce platelet activation and blood coagulation, stimulate cell immune response and tissue regeneration.

For Topical acne treatment was referred from a clinical practice guideline for acne which was established by a team of dermatologists and approved for publishing by Dermatological Society of Thailand(DST).

In this research, comparing efficacy of non-thermal plasma device combined with topical treatment versus topical treatment alone for moderate facial acne vulgaris in Thais**.**

Objective

To examine the efficacy of plasma technology in enhancing treatment of acne vulgaris (in term of duration) compared with the topical treatment of acne.

Materials and Methods

Thirty Thai volunteers were enrolled and had moderate facial acne vulgaris at both sides of face. Randomly assigned for treatment by non-thermal plasma device combined with topical treatment and topical treatment alone in each sides. All volunteers received topical treatment for applying full-face. Half of face would receive non-thermal plasma once a week for 4 weeks. Clinical evaluation was done at week 4, 8, 12 by using photographs of VISIA complexion analysis, acne grading and acne lesion counting. Side effects and satisfaction were assessed by questionnaire.

Statistic for Data Analysis

The primary clinical endpoint were acne grade (mild, moderate and severe) and lesion count (non-inflammatory and inflammatory lesions), compared before and after treatment (at week 4,8,12) by non-thermal plasma combined with topical treatment and topical treatment alone, using Wilcoxon sign rank test and ANOVA. In addition, acne grade and lesion count were compared between two groups of treatment, using Chi-square test and independent T-test. Similar comparison was made for secondary endpoint (adverse effects and satisfaction score). The confidential level was specified at 95%. Significance levels for all analyses were set at p-value < .05.

Results

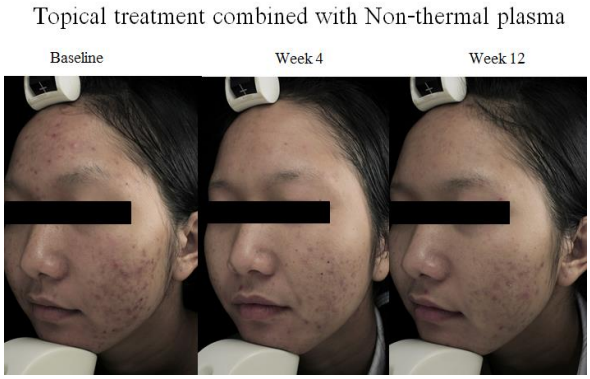


Figure 1. These were the pictures of case in topical treatment with non-thermal plasma group.

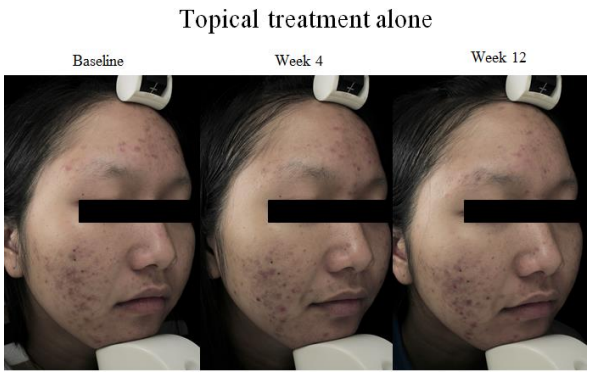


Figure 2. These were the pictures of case in topical treatment group.

Twenty eight volunteers completed the study. Both sides of face had statistically significant decreased from baseline in acne grade and acne lesion count. (Figure 3, 4) Acne grade before treatment was 2 and after treatment until week 12 was 1.14 ± 0.35(83% of the patients in this group turn to mild acne after treating for 12 weeks) in side of treatment by non-thermal plasma combined with topical treatment (figure 5) while acne grading before treatment was 2 and after treatment until week 12 was 1.55 ± 0.51 (43% of this group turn to mild acne after treating for12 week) in side of treatment by topical treatment alone. (Figure 6)

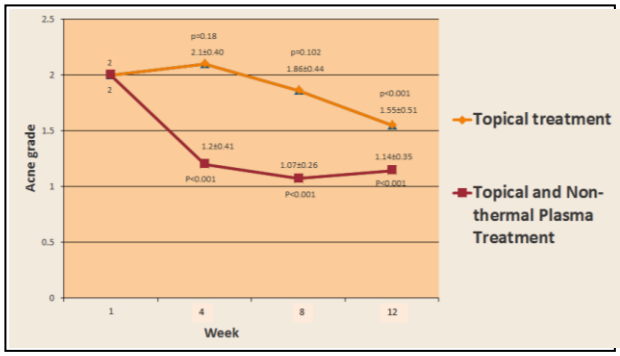


Figure 3. Linear graph of comparing acne grade of before and after treatment (at week 4, 8 and 12) by non-thermal plasma combined with topical treatment and topical treatment alone.

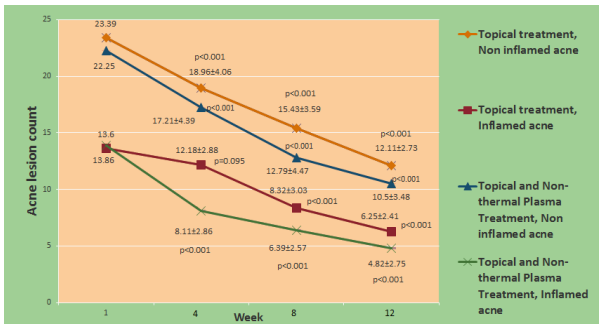


Figure 4. Linear graph of comparing acne lesion count of non-inflamed and inflamed acnes before and after treatment (at week 4, 8 and 12) by non-thermal plasma combined with topical treatment and topical treatment alone.

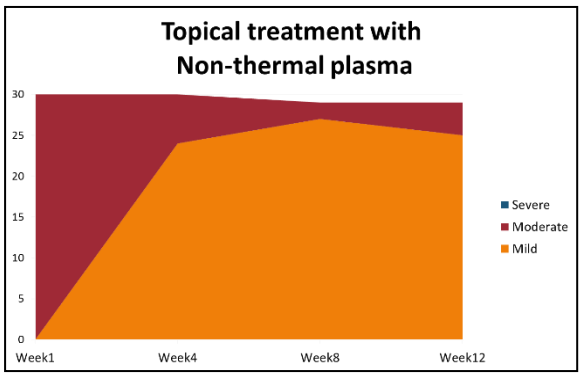


Figure 5. This area chart showed 83% of the patients turn to mild acne after treating with topical treatment with non-thermal plasma for 12 weeks. Some patient dropouts could be seen in 8th week and 12th week.

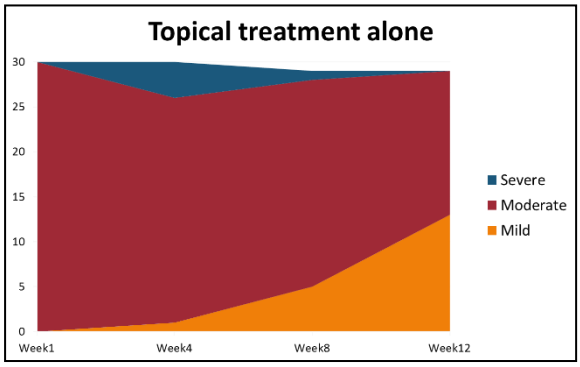


Figure 6. This area chart showed 43% of the patients turn to mild acne after treating with topical treatment for 12 weeks. Some patient dropouts could be seen in 8th week and 12th week.

Acne lesion count for non-inflamed and inflamed acne before treatment were 22.25±4.73 and 13.86±2.37, respectively and after treatment until week 12 were 10.5±3.48 and 4.82±2.75, respectively in side of treatment by non-thermal plasma combined with topical treatment. Acne lesion count for non-inflamed and inflamed acne before treatment were 23.39±5.30 and 13.68±2.45, respectively and after treatment until week 12 were 12.11±2.73 and 6.25±2.41 in side of treatment by topical treatment alone. (Figure2)

The side of treatment by non-thermal plasma combined with topical treatment had better outcome than topical treatment alone in term of duration, there was statistically significant difference at week 4, 8 and 12 when evaluated by comparing acne grade (p-value < 0.01). (Figure5)

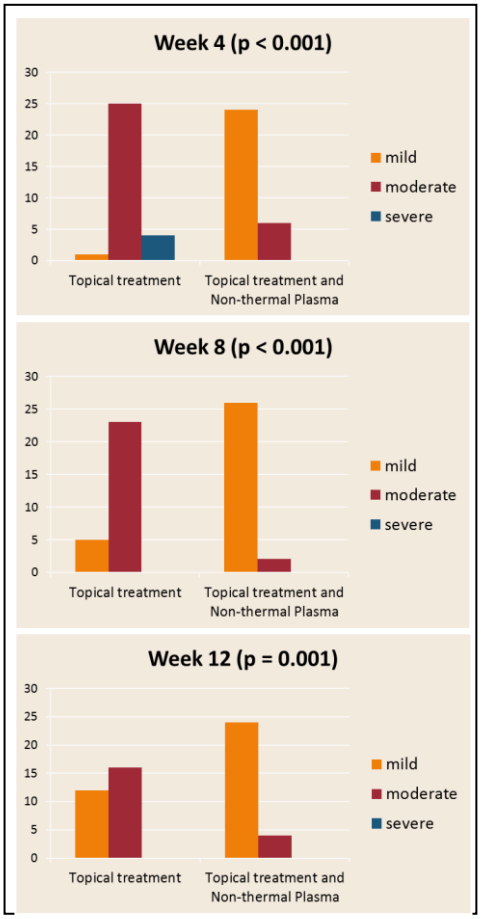


Figure 5. To compare acne grade (mild, moderate and severe) at week 4,8 and 12 between the treatment by non-thermal plasma combined with topical treatment and topical treatment alone.

In addition, acne lesion count also showed statistically difference in inflamed acne at week 4,8 and 12 (p-value < 0.01, p-value = 0.013, p-value = 0.044). Non-inflamed acne only at week 8 showed statistically significant difference (p-value = 0.018). (Table1)

Table 1. To compare acne lesion count (non-inflamed acne and inflamed acne) at week 4,8,12 between the treatment by non-thermal plasma combined with topical treatment and topical treatment alone

	Intervention	Mean	SD	p value
Lesion count week 4 (Non-inflamed acne)	Topical treatment	19.27	4.1	**0.183**
	Topical treatment and Non-thermal plasma	17.73	4.68	
Lesion count week 4 (Inflamed acne)	Topical treatment	12.63	3.29	**<0.01**
	Topical treatment and Non-thermal plasma	8.13	2.76	
	Intervention	**Mean**	**SD**	**p value**
Lesion count week 8 (Non-inflamed acne)	Topical treatment	15.43	3.59	**0.018**
	Topical treatment and Non-thermal plasma	12.79	4.47	
Lesion count week 8 (Inflamed acne)	Topical treatment	8.32	3.03	**0.013**
	Topical treatment and Non-thermal plasma	6.39	2.57	
	Intervention	**Mean**	**SD**	**p value**
Lesion count week 12 (Non-inflamed acne)	Topical treatment	12.11	2.73	**0.06**
	Topical treatment and Non-thermal plasma	10.5	3.48	
Lesion count week 12 (Inflamed acne)	Topical treatment	6.25	2.41	**0.044**
	Topical treatment and	4.82	2.75	

No statistically significant difference of adverse effects; dry skin, scaly skin, acne flare (p-value = 0.609, p-value = 0.803 , p-value = 0.889) except erythema (p-value = 0.015) between treatment by non-thermal plasma combined with topical treatment and topical treatment alone. (Table 2) For satisfactory score, treatment by non-thermal plasma combined with topical treatment was showed to be statistically significant when compared to treatment by topical treatment alone (p-value = 0.024). (Table 3)

Table 2. Evaluation for adverse effects

	Topical treatment	**Topical treatment and Non-thermal Plasma**	**p value between group**
Dry skin	21 (75%)	23 (82.1%)	
Scaly	13 (46.4%)	12 (42.9%)	>0.05
Acne flare	6 (21.4%)	5 (17.9%)	
Erythema	0	5 (17.9%)	0.019

Table 3. Evaluation for satisfaction score

	Topical treatment	**Topical treatment with Non-thermal plasma**	**p value between group**
Worse	0	0	
Same	0	0	
Mild satisfaction	0	0	
Moderate satisfaction	7 (25%)	2 (7.1%)	0.04
Good satisfaction	11 (37.3%)	7 (25%)	
Excellent satisfaction	10 (35.7%)	19 (67.9%)	

Discussion

From the research, we found that the acne grade and acne lesion count especially Inflamed acne were decreased by non-thermal plasma combined with topical treatment had better outcome (in term of duration) than topical treatment alone which could be explained by pathogenesis of acne. The benefits of non-thermal plasma were P. acne sterilization, deceasing inflammation, sebum production and removing keratin plugs and the dead keratin layer, confirmed with the researches in the past. Dr. Wasini Techawatthanawisan from MFU founded that the inflammatory acnes were reduced significantly by non-thermal plasma device in her pilot study . (Techawatthanawisan W., 2011).

In addition, Dr. Treenuch Kundilokchai showed that non-thermal plasma could reduce the facial sebum production in the week 4,8 of appling non-thermal plasma once a week for 4 week.(Kundilokchai T.,2012). Moreover the previous study on the direct DBD plasma of Chutsirimongkol showed that there were an effect of sterilization, stimulation wound healing and tissue regeneration.(Chutsirimongkol C., et al. 2014).

Although we used topical treatment in both side of face, the duration of this study was quite short for the best result of the topical retinoid in some volunteers and few patients also had some acne flare in the few weeks after starting the study. After we stopped both interventions, we found that the half face which used to apply the plasma would occur the inflamed acne less than the other side.

For the adverse effects, applying benzoyl peroxide and topical retinoid, dry and scaly skin could be found due to sebo-suppressive effect (Gloor M. et al. Arch Dermatol Res. 1980) and decreasing sebum production (tends to peak within the first month of treatment and diminishes thereafter), respectively.(Chien L. A., et al. 2012).

Only 5 person(8.3%) of treatment by non-thermal plasma combined with topical treatment group had erythema after each session of non-thermal

plasma.The other adverse effects resembled between two groups; dry skin, scaly skin and acne flare because of topical treatment.

For satisfactory score, treatment by non-thermal plasma combined with topical treatment was showed to be statistically significant when compared to treatment by topical treatment alone. It might be the efficacy in the treatment by non-thermal plasma combined with topical treatment had better result than topical treatment alone.

Summary

Non-thermal plasma combined with topical treatment was more efficient in reducing acne grade and acne lesion count in term of duration of treatment than using topical treatment alone. Satisfaction was also much more in combined treatment group. For the further studies, energy setting in each skin type should be studied for making the best therapeutic outcomes in each patient, moreover, other severities of acne should be evaluated efficacy of treatment.

References

Techawatthanawisan, W. (2011). An efficacy assessment of a dielectric-barrier-discharge plasma device for adjuctive therapy of acne vulgaris: a pilot study. **Chiang-Rai. Mae Fah Laung University.**

Kundilokchai, T. (2012). The effectiveness of a non-thermal plasma device for reducing sebum production on oily face. **Chiang-Rai. Mae Fah Laung University.**

Chutsirimongkol, C., Boonyawan, D., Polnikorn, N., Techawatthanawisan, W. & Kundilokchai, T. (2014). Non-Thermal Plasma for Acne Treatment and Aesthetic Skin Improvement. **Plasma Medicine, 4(1-4),** 79-88.

Yentzer BA, et al. (2009). Do topical retinoids case acne to flare. **J Drug Dermatol, 8(9),** 799-801.

Gloor M, et al. (1980). Cytokinetic studies on the sebo-suppressive effect of drugs using the example of benzoyl peroxide. **Arch Dermatol Res, 267(1),** 97-9.

Chien, L. A., Voorhees, J. J., Kang, S. (2012). Topical retinoids. **Fitzpatrick's Dermatology in General Medicine**(8th ed., pp. 2665-2672). New York: McGrawhill.

A Comparison of the Efficacy between Topical 5% Benzoyl Peroxide and Topical 0.05% Tretinoin in the Treatment of Trichostasis Spinulosa

Pimthong Pakawatsuntorn[1*], Tanomkit Pawcsuntorn[2]

[1]*Student in Master of science in Dermatology program, School of Anti-aging and regenerative medicine, Mae Fah Luang University, 38/11-13 Asoke Place Building, Sukhumvit 21, Klong Toey Nua, Wattana, Bangkok, 10110, Thailand*
[2]*Advisor, School of Anti-aging and regenerative medicine, Mae Fah Luang University, 38/11-13 Asoke Place Building, Sukhumvit 21, Klong Toey Nua, Wattana, Bangkok, 10110, Thailand*
**Corresponding author. E-mail: p.pakawatsuntorn@gmail.com*

Abstract

Trichostasis spinulosa characterized by hyperkeratotic follicular papules resulting from the follicular retention of vellus hairs, is a relatively common disease that cause a cosmetic disturbance. A variety of treatment modalities have been used such as topical retinoids, keratolytic drugs, adhesive strips, IPL and laser. Although no any gold standard treatment available, topical retinoids is the one that found effective. Topical benzoyl peroxide has keratolytic activity, antimicrobial activity, and anti-inflammatory activity, which may has a role in the treatment of trichostasis spinulosa. The objective of the study is to compare the efficacy between topical 5% benzoyl peroxide and topical 0.05% tretinoin in the treatment of trichostasis spinulosa. Thirty seven volunteers who had trichostasis spinulosa and met the inclusion criteria were enrolled and randomly assigned topical 5% benzoyl peroxide and topical 0.05% tretinoin to each alae nasi. Photographs were taken by a digital microscope at baseline, 4th, 8th and 12th week, an independent physician counted the lesion of trichostasis spinulosa from the digital microscope photographs. Among thirty seven volunteers enrolled, thirty four subjects completed the study. The subjects were female (20/34, 58.82%) and male (14/34, 41.18 %). The average participant age was 32.97 ± 10.12 years old. The results revealed both topical 5% benzoyl peroxide and topical 0.05% tretinoin can reduce the number of trichostasis spinulosa at 4th, 8th and 12th week. There were no statistically significant in the efficacy between topical 5% benzoyl peroxide and topical 0.05% tretinoin. Both treatment show no severe complication, only minimal adverse effects are itching, redness, dry and scale.

Keywords: benzoyl peroxide/ tretinoin/ trichostasis spinulosa

Introduction

Trichostasis spinulosa is a relatively common disease characterized by hyperkeratotic follicular papules resulting from the follicular retention of vellus hairs. This skin disorder is not harmful but can cause a cosmetic disturbance. The ethiology are external irritation resulting in hyperkeratosis and plugging that prevent extrusion of normal lanugo hair, disturbance in sebum production, anatomical abnormalities of hair, microorganism, etc.

A variety of treatment modalities have been used such as topical retinoids, keratolytic drugs, adhesive strips, IPL and laser. Although no any gold standard treatment available, topical retinoids is the one that found effective.

Topical benzoyl peroxide is the drug that widely used to treat acne, its safety and efficacy are unquestionable. According to its property in keratolytic activity, antimicrobial activity and anti-inflammatory activity, the topical benzoyl peroxide may has a role in the treatment of trichostasis spinulosa.

Objective

To compare the efficacy between topical 5% benzoyl peroxide and topical 0.05% tretinoin in the treatment of trichostasis spinulosa.

Materials and Methods

Research methodology

Thirty seven volunteers aged above eighteen who had trichostasis spinulosa and met the inclusion criteria, after obtaining their consent, were enrolled and randomly assigned by block randomization to use topical 5% benzoyl peroxide twice daily before washing the face and topical 0.05% tretinoin once daily at night to each alae nasi for the first eight weeks, from the 8th to 12th week the usage was twice a week on Monday and Thursday. The exclusion criteria in this study were women who were pregnant or breastfeeding, participants who had trichostasis spinulosa treated in the past 3 months or received IPL/laser treatment in the past 1 year, having extensive sun exposure in the past 1 week, having skin lesion at the alae nasi, or having uncontrolled disease such as diabetes mellitus, kidney

disease, AIDS, etc. The primary end-point for efficacy evaluation was based on the number of trichostasis spinulosa evaluated by photographs taken by a digital microscope at baseline, 4th, 8th and 12th week. The number of lesions was counted by using the nose pore pack that has six holes, diameter 0.5 centimeters each, three holes on the left side and other three on the right side. It was placed one centimeter above nasal septum. An independent physician counted trichostasis spinulosa from the digital microscope photographs. The secondary end-point was the improvement evaluated by subjects, subjects' satisfaction and side effects.

Statistic for Data Analysis

Volunteers' research profile data using descriptive statistical analysis to provide descriptive information, such as percentage, means, modes, medians and standard deviations. Comparison of mean of numbers of trichostasis spinulosa between topical 5% benzoyl peroxide side and topical 0.05% tretinoin side use pair t-test. Comparison of mean of numbers of trichostasis spinulosa between baseline, 4th, 8th and 12th week uses Repeated Measure ANOVA. Comparison of improvement evaluated by patients uses Wilcoxon sign rank test. Comparison of satisfaction evaluated by patients use Wilcoxon sign rank test. The side effects of both drugs use descriptive statistical analysis to provide descriptive information. The researcher did the following at significance of p-value < 0.05

Results

Among thirty seven volunteers enrolled, thirty four subjects completed the study. The subjects were female (20/34, 58.82%) and male (14/34, 41.18 %). The average participant age was 32.97 ± 10.12 years old.

The results revealed both topical 5% benzoyl peroxide and topical 0.05% tretinoin can reduce the number of trichostasis spinulosa at 4th, 8th and 12th week. There were no statistically significant in the efficacy between topical 5% benzoyl peroxide and topical 0.05% tretinoin.

There were no different in improvement evaluated by patients. For patients' satisfactory evaluation, topical 0.05% tretinoin was more convenient to use, but in the continue-to-use evaluation and overall satisfaction, there were no different.

Both treatment show no severe complication, only minimal adverse effects are itching, redness, dry and scale.

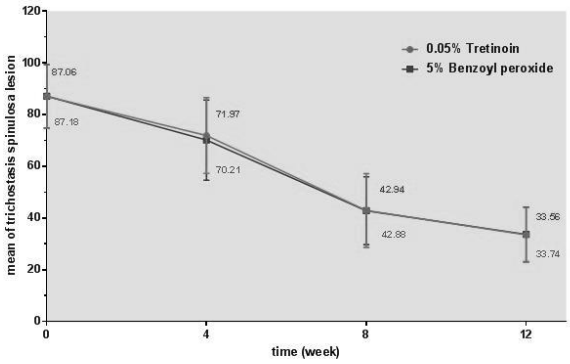
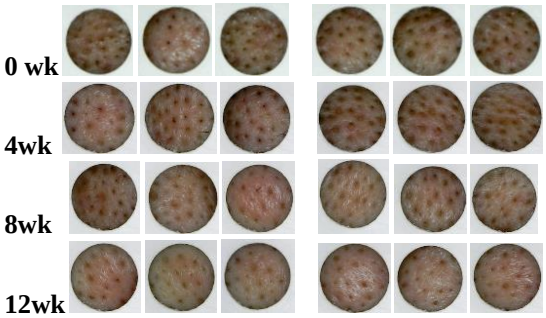


Figure 1 Linear graphs compared difference mean of lesions between topical 5% benzoyl peroxide treatment side and topical 0.05% tretinoin treatment side at baseline, 4th, 8th and 12th week.



Left topical 0.05% tretinoin, Right topical 5% benzoyl peroxide

Figure 2 Both topical 5% benzoyl peroxide and topical 0.05% tretinoin can reduce the number of trichostasis spinulosa at 4th, 8th and 12th week.

Discussion

From the research, we found that trichostasis spinulosa was effectively treated by topical 5% benzoyl peroxide and topical 0.05% tretinoin equally. These two drugs can reduce the number of trichostasis spinulosa at 4th, 8th and 12th week. This is consistent with the finding in previous study such as in Otto H. et al's (Otto, 1973) that topical tretinoin can reduce the number of trichostasis spinulosa by 75-90% in 6-8 weeks and in Narumon's study (Narumol, 2011) that topical tretinoin can reduce the number of trichostasis spinulosa within 8 weeks. In addition, the finding is also consistent with the property of benzoyl peroxide in keratolytic activity, antimicrobial activity and anti-inflammatory activity with serves a role in pathophysiology of trichostasis spinulosa.

The research outcome was evaluated by photographs taken by the digital microscope which is more sensitive and accurate than by photographs from a camera or by clinical diagnosis alone.

For satisfactory score, topical 0.05% tretinoin was more convenient to use because the usage is once daily but the usage of 5% benzoyl peroxide was twice a day

and before washing the face, but in the continue-to-use evaluation and overall satisfaction, there were no different, this is might because these two drugs are equally effective.

Both treatment shows no severe complication, only minimal adverse effects are itching, redness, dry and scale which decrease after continuous using, conform with the previous research that the side effects of these drugs will decrease after continuous using.

Conclusions

There is no different in the efficacy between topical 5% benzoyl peroxide and topical 0.05% tretinoin. Both drugs has efficacy and are safe in the treatment of trichostasis spinulosa.

References

Antille et. (2003). Vitamin A exerts a photoprotective action in skin by absorbing ultraviolet B radiation. J Invest Dermatol. 121(5):1163-7.

Ashraf Badawi, Mouhamad Kashmar. (2011). Treatment of trichostasis spinulosa with 0.5- millisecond pulsed 755-nm alexandrite laser. Lasers in Medical Science, Volume 26, Issue 6, pp 825-829

Babamiri K1, Nassab R. (2010). Cosmeceuticals: the evidence behind the retinoids. Aesthet Surg J. 30(1):74-7.

Baumann, L. (2009). Retinoids. Cosmetic dermatology: Principles and practice. 256-262.

Brogden RN, Speight TM, Avery GS. (1974). Benzoyl peroxide acne lotions: an independent report. Drugs. 8(6):417-21.

Burkhart CN, Burkhart CG. (2006). Antibacterial properties of benzoyl peroxide in aerobic and anaerobic conditions. International Journal of Dermatology. 45(11):1373-1374.

Chung TA, Lee JB, Jang HS, Kwon KS, Oh CK.(1998) A clinical, microbiological, and histopathologic study of trichostasis spinulosa. J Dermatol. 25:697–702.

Cove H., Holland KT. The effect of benzoyl peroxide on cutaneous micro-organism in vitro. J Appl Bacteriol. 54:379-382.

Cunliffe WJ, Burke B. (1982). Benzoyl peroxide:lack of sensitization. Acta Derm Venereol. 62: 458–459.

Darlenski R, Surber C, Fluhr JW. (2010). Topical retinoids in the management of photodamaged skin: from theory to evidence-based practical approach. Br J Dermatol. 163(6):1157-65.

Deshmukh SD, Anand M, Yadav GE, Joshi AR. (2011). Trichostasis spinulosa presenting as itchy papules in a young lady. Int J Trichology. 3(1):44-5.

Emil A.Tanghetti. Karl F. Popp.(2009) A Current Review of Topical Benzoyl Peroxide: New Perspectives on Formulation and Utilization. Dermatol Clin. 27:17–24.

Emory Ladany. (1954) Trichostasis Spinulosa. The Journal of Investigative Dermatology 23:33–41.

Fulton J. E., et al. (1974). Studies on the Mechanism of Action of Topical Benzoyl Peroxide and Vitamin A Acid in Acne Vulgaris. Journal of Cutaneous Pathology. 1(5):191–200

Gans EH, Kligman AM. (2002). Comparative efficacy of clindamycin and benzoyl peroxide for in vivo suppression of Propionibacterium acnes. J Dermatolog Treat.13:101–10.

Gündüz, Özge, Aytekin, Aslı. (2012). Trichostasis Spinulosa Confirmed by Standard Skin Surface Biopsy. International Journal of Trichology. 4(4): 273-274.

Harvey J.,et al. (1997). A physiologically based pharmacokinetic model for retinoic acid and its metabolites. JAAD. 36(3): S77–S85

Heather Pickett, Scott Wiltz. (2011). Adapalene/Benzoyl Peroxide (Epiduo) for Acne Vulgaris. American Family Physician, 1490-1491.

Janjua SA, McKoy KC, Iftikhar N. (2007). Trichostasis spinulosa: Possible association with prolonged topical application of clobetasol propionate 0.05% cream. Int J Dermatol. 46:982–5.

Kailasam, Kamalam & Thambiah. (1979). Trichostasis spinulosa. International Journal of Dermatology. 18(4):297–300

Kigman A. M., Leyden J.J., Stewart R. (1977). New uses for benzoyl peroxide: a broad-spectrum antimicrobial agent. Int J Dermatol. 16(5):413-7.

Kikkeri Narayanasetty Naveen,Suraj R. Shetty. (2014). Trichostasis spinulosa: An overlooked entity. Indian Dermatol Online J. 5: S132–S133.

Kligman AM. (1995). Acne vulgaris: tricks and treatments. Part II. The benzoyl peroxide saga. Cutis. 56(5):260–261

Krivda, S.J. & vonHilsheimer, G. (2010). Trichostasis spinulosa. Retrieved August 11, 2015,from http://emedicine.medscape.com/article/1071782-overview

Mills OH Jr, Kligman AM, Pochi P, Comite H. (1986). Comparing 2.5 %, 5 %, and 10 % benzoyl peroxide on inflammatory acne vulgaris. Int J Dermatol. 25: 664–667.

Nacht S, Yeung D, Beasley JN, et al. (1981). Benzoyl peroxide: percutaneous penetration and metabolic disposition. J Am Acad Dermatol. 4(1):31-7.

Narumon Teeranulak. (2011). A comparison of the efficacy between 2 % Salicylic acid cream and 0.05 % Tretinoin cream in the treatment of Trichostasis spinulosa. retrieve from www.mfu.ac.th/school/anti-aging/File_PDF/Research_PDF55/6.pdf

Nascimento LV, Guedes ACM, Magalhaes GM, et al. (2003). Single-blind and comparative clinical study of the efficacy and safety of benzoyl peroxide 4% gel (BID) and adapalene 0.1% Gel (QD) in the treatment of acne vulgaris for 11 weeks. J. Dermatolog Treat. 14(3):166-71.

Navarini AA, Ziegler M, Kolm I, Weibel L, Huber C, Trüeb RM. (2010). Minoxidil-induced trichostasis spinulosa of terminal hair. Arch Dermatol. 146:1434–5.

Otto H. Mills Jr., MA; Albert M. Kligman. (1973). Topically Applied Tretinoin in the Treatment of Trichostasis Spinulosa. Arch Dermatol. 108(3):378-380

Rendon MI, Gaviria JI. (2005). Review of skin-lightening agents. Dermatol Surg. (31):886–9

Sidwell RU, Francis N, Bunker CB. (2006). Diffuse trichostasis spinulosa in chronic renal failure. Clin Exp Dermatol. 31:86–8.

Simonart T. (2012). Newer approaches to the treatment of acne vulgaris. Am J Clin Dermatol. 13(6):357-64

Strobos MA, Jonkman MF. (2002) Trichostasis spinulosa: itchy follicular papules in young adults. Int J Dermatol. 41(10):643-6.

Toosi S, Ehsani AH, Noormohammadpoor P, Esmaili N, Mirshams-Shahshahani M, Moineddin F. (2010). Treatment of trichostasis spinulosa with a 755-nm long-pulsed alexandrite laser. J Eur Acad Dermatol Venereol. 24:470–473.

Van Hoogdalem EJ. (1998). Transdermal absorption of topical anti-acne agents in man; review of clinical pharmacokinetic data. J Eur Acad Dermatol Venereol. 11(Suppl 1):S13–9**.**

Waller JM, Dreher F, Behnam S, et al. (2006). 'Keratolytic' properties of benzoyl peroxide and retinoic acid resemble salicylic acid in man. Skin Pharmacol Physiol. 19(5):283-9.

Webster GF. (1998). Acne and rosacea. Med Clin North Am. 82:1145–1154.

Wepierre J et al. (1986). Distribution and dissociation of benzoyl peroxide in cutaneous tissue after application on skin in the hairless rat. Int J Cosmet Sci. 8 (3): 97-104..

Wolf-Ingo Worret, Joachim W. Fluhr. (2005). 19th WHO Model List of Essential Medicines. WHO.

A Comparison of Wound Healing between Aloe Vera Cream and Placebo After Melasma Treatment with Laser

NiranWanmuang[1*], and PaisalRummaneethorn[2]

[1]*Student in program Master of Science Program in Dermatology, School of Anti-aging and regenerative medicine, Mae FahLuang University, 38/11-13 Asoke Place Building, Sukhumvit 21, KlongToeyNua, Wattana, Bangkok, 10110, Thailand*

[2]*Advisor, School of Anti-aging and regenerative medicine, Mae FahLuang University, 38/11-13 Asoke Place Building, Sukhumvit 21, KlongToeyNua, Wattana, Bangkok, 10110, Thailand*

[*]*Corresponding author. E-mail: sam_gullas@hotmail.com*

Abstract

Fractional Q switch laser has been proven effective for the treatment of melasma, photodamage and wrinkles. A key factor for best result with laser treatment may influence some postprocedural damage skin such as erythematous, swelling, and ablative skin. Currently, there is no standard care for postprocedural laser treatment. Common practice for postprocedural laser are gradually healing or application topical steroid, antibiotic. But drugs have the potential to cause allergy, side effect, and increase drug resistance. Now a day herbal is an excellent and alternative treatment condition such as burn, eczema, etc. aloe vera is one of natural vegetarian sources of multi phytochemicals for healthy skin.

Keywords:Fractional Q- switch Nd:YAG/ Melasma/Aloe vera/ Placebo

Introduction

Fractional laser which demand high laser intensities in nanosecond pulses an optional treatment for melasma. Recently, a new generation of these laser Q-switch 1064-nm neodymium:YAG (Nd:YAG) these lasers featuring higher power and fractionated beams have been introduced for non-ablative skin remodeling and rejuvenation that creates a 5x5 matrix with 25 microscopic holes and high power density per pixel

Melasma is the skin hyperpigmentation irregular well demarcated common found on the upper cheek, nose and forehead, because of multifactor including sunlight, hormones, drug (HRT) and genetic etc. particularly common in women, especially pregnant women

Aloe vera is plant grows wild in tropical climates, it contain phytochemicals for bioactivities, such as acetylated mannans, antharquinone and aloctin A,B is an excellent for burns, eczema and wound healing

In this research, the study has compared aloe vera cream and placebo cream after melasma treatment with laser. And evaluation efficacy from clinical outcome by physician, measuring Mean Melanin Index by Mexameter MX 18, tranepidermal water loss (TEWL) by Tewameter TM300 and photographs by VisiofaceQuickround, side effects and subjects satisfaction.

Objective

To compare the efficacy of aloe vera cream and placebo cream after melasma treatment with Q switch laser.

Materials and Methods

Research methodology

30 subjects, (aged range 25-50 years) with melasma were randomly applied aloe vera cream and placebo cream split face on postprocedural melasma area twice a day for 3 days with follow-up once day, The measuring Mean Melanin Index by Mexameter MX 18, tranepidermal water loss (TEWL) by Tewameter TM300, photographs by VisiofaceQuickround and clinical grading by physician, Both values and adverse side effects were performed at the start and the end of the study, Subjects satisfaction were assessed by questionnaires.

Statistic for Data Analysis

Subject' research profile data using descriptive statistical analysis to provide descriptive information, such as percentage, means, modes, medians and standard deviations. Comparison of mean of melanin index and tranepidermal water loss (TEWL) between aloe vera cream and placebo cream use Mann-Whitney U-test. Comparison of mean of melanin index and tranepidermal water loss (TEWL) between baseline, 1st,

2nd, and 3rdday uses Wilcoxon sign rank test. Comparison of improvement evaluated by patients uses Wilcoxon sign rank test. Comparison of satisfaction evaluated by patients use Wilcoxon sign rank test. The side effects of both drugs use descriptive statistical analysis to provide descriptive information. The researcher did the following at significance of p-value < 0.05

Results

Thirty subjects completed the study. The study found that aloe vera cream and placebo cream statistically significant difference (p<0.001) decrease tranepidermal water loss in both groups when evaluated by Tewameter, but comparing between two groups no statistically significant difference (p<0.817) (e.g. Figure 1). And shown statistically significant difference (p<0.001) decrease Mean Melanin Index in both groups when evaluated by Mexameter, but comparing between two groups no statistically significant difference (p<0.405) (e.g. Figure 2). Clinical grading and irritation assessments showed no statistically significant difference between two groups at any time point. Subjects two groups no adverse side effects with aloe vera cream and placebo cream. For satisfactory score was no statistically significant difference between two groups.

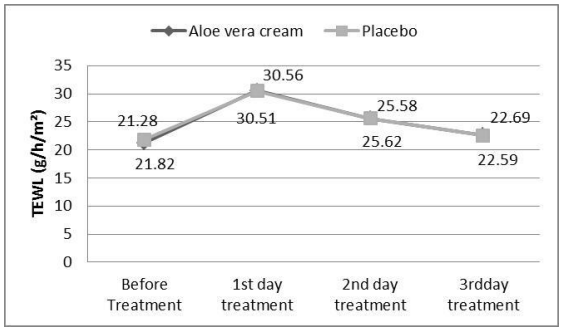


Figure 1. Linear graphs compared difference TEWL (g/h/m²) between aloe vera cream and placebo

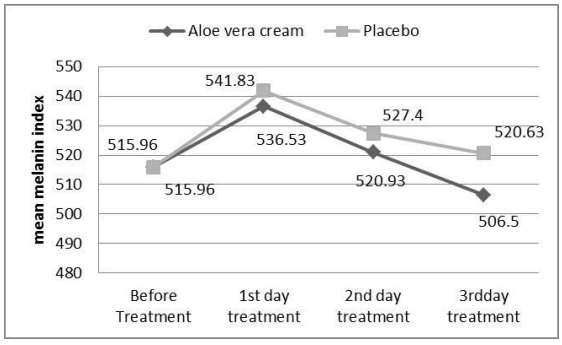


Figure 2. Linear graphs compared difference mean melanin index between aloe vera cream and placebo

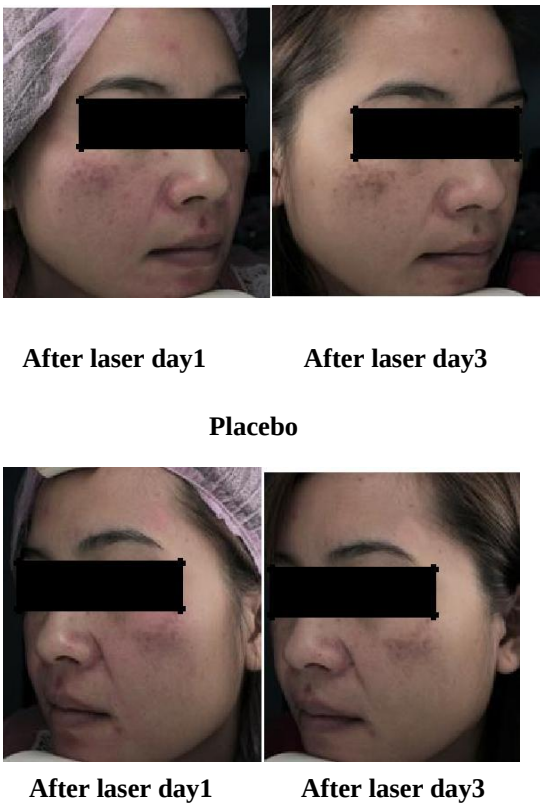


Figure 3. To compare photographs of digital camera between the treatment by aloe vera cream and placebo in after laser day1 and day3 treatment.

Discussion

From the research, we found that the subjects in the group applied aloe vera cream and the group applied placebo cream, both have been shown statistically significant difference in decrease tranepidermal water loss and Mean Melanin Index.

The research outcome that the aloe vera cream and placebo cream statistically significant difference when evaluated with measuring tranepidermal water loss by Tewameter and measuring Mean Melanin Index by Mexameter after melasma treatment with laser Q-switch 1064-nm neodymium:YAG (Nd:YAG) during three day postprocedural, but comparing between two groups no statistically significant difference. Clinical grading and irritation assessments showed no statistically significant difference between two groups at any time point.

For satisfactory score was no statistically significant difference between two groups.Therefore, the research result in efficacy, side effects, and satisfaction in treatment has supported the research assumption as mentioned in the beginning

Conclusion

Postprocedural treatment with aloe vera cream and placebo cream demonstrated equivalent efficacy on wound healing. Therefore aloe vera cream is an alternative treatment for wound healing after melasma treatment with laser Q-switch 1064-nm neodymium:YAG (Nd:YAG).

Reference

Baumann, L. (2008). Cosmetic Dermatology In. K. Wolff, L. A. Goldsmith, S. I. Katz, B. A. Gilchrest, A. S. Paller& D. J. Leffell (Eds.), Fitzpatrick's dermatology in general medicine (Vol. 2, 7th ed., pp. 2357-2359). New York: McGraw-Hill.

Baumann, L. (2009). Moisturizing agents. In Cosmetic dermatology: Principles and practice (2nd ed., p. 274). China: McGraw-Hill.

Goerge, T., Peukert, N., Bayer, H. &Rütter, A.(2008). Ablative fractional photothermolysis-novel step in skin resurfacing.Med Laser Appl, 23(2), 93-98.

Goldman, M. P., Roberts, T. L., Skover, G., Lettieri, J. T. & Fitzpatrick, R. E. (2002). Optimizing wound healing in the face after laser abrasion. J Am AcadDermatol, 46(3),399-407.

Rogiers, V. & the EEMCO group.(2001). EEMCO Guidance for the Assessment of Transepidermal Water Loss in Cosmetic Sciences. Skin PharmacolAppl Skin Physiol,14(2), 117-128

Acute Cadmium Exposure Induces the Up-Regulation of Proinflammatory Cytokines without the Reduction of Glial Glutamate Transporter Expression in Human Astrocytes

Suttinee Phuagkhaopong[1], Thitima Kasemsuk[1], Dusadee Ospondpant[1], Sirada Srihirun[2], Kulathida Chaithirayanon[3], Nathawut Sibmooh[1], Pornpun Vivithanaporn[1*]

[1]*Department of Pharmacology, Faculty of Science, Mahidol University, Bangkok 10400, Thailand*
[2]*Department of Pharmacology, Faculty of Dentistry, Mahidol University, Bangkok 10400, Thailand*
[3]*Department of Anatomy, Faculty of Science, Mahidol University, Bangkok 10400, Thailand*
**Corresponding author. E-mail: pornpun.viv@mahidol.ac.th*

Abstract

Chronic exposure to cadmium has been linked to neurodegenerative diseases and neurobehavioral defects. Previous studies of cadmium toxicity in the central nervous system focused on neurobehavior deficit in animal models and neuronal cell death. Few studies on astrocytesindicated oxidative stress as the underlying cause of astrocyte death. However, cadmium also induces inflammatory response by promoting the release of proinflammatory cytokines in lung tissues and leukocytes. In addition to oxidative stress, neuroinflammation and glutamate-induced excitotoxicity are recognized as major causes of neurological disorders. In the present study, we investigated the effects of cadmium on the expression of proinflammatory cytokines and glutamate transporter as well as its underlying mechanism on human astrocytes. Here we showed that exposure of astrocytes to cadmium at 30 μM, which reduced cell viability to 60% and 40% at 6 hours and 24 hours, respectively, promoted the mRNA expression of proinflammatory cytokines including TNF-α, IL-1β and IL-6, while the expression of excitatory amino acid transporter 2 (EAAT2) was not changed. Cadmium strongly promoted the secretion of IL-6 in a time-dependent manner and the excessive release of IL-6 could be suppressed by an NF-κB inhibitor. All of these results suggested that cadmium-induced astrocytic death could be a result from the up-regulation of proinflammatory cytokines including TNF-α, IL-1β and IL-6 through the NF-κB pathway. Additionally, our results suggested that the reduction of glutamate uptake may not be responsible for the acute toxicity of cadmium. This information indicated that the inhibition of NF-κB pathway could be a novel approach for preventing cadmium-induced neuroinflammation and neurological disorders.

Keywords: TNF-α, IL-1β, IL-6, EAAT2, NF-κB

Introduction

Toxicities of cadmium to kidneys, liver and cardiovascular system are well known; however, recent evidences showed that cadmium is also toxic to the central nervous system (CNS). Cadmium is released to the air from the burning of fossil fuels and cigarette smoking. Another route of cadmium exposure is via ingestion [1]. Cadmium accumulates in many organs and tissues, including kidneys, liver, lungs and brain. Cadmium increases blood-brain barrier permeability and penetrates into the brain [2]. The maximum level of cadmium detected in the brain is approximately 30 μM [3]. An epidemiological study showed the association of cadmium exposure with learning disability in children and neurodegenerative diseases [4,5,6]. High concentration of cadmium in the CNS has been shown to be involved in brain cell damage and cell loss by various mechanisms, including the mitochondrial dysfunction, oxidative stress and glutamate-induced excitotoxicity [7,8,9,10]. In the peripheral system, cadmium also induces inflammatory response by promoting the release of proinflammatory cytokines. Cadmium significantly induced interleukin(IL)-1, tumor necrosis factor(TNF)-α, macrophage inflammatory protein(MIP)-2 and IL-6 secretion from human type 2 epithelial cells, alveolar macrophage and human lung cells [11,12]. Cadmium activated nuclear factor (NF)-κB in THP-1 human monocytic leukemia cells [13] and human lung epithelial cells [14] and ERK1/2 MAPK in

human airway epithelial cells [15].

The major role of astrocytes is the uptake of glutamate by EAAT2. Dysfunction of astrocytic glutamate transporters induced by toxicants and infection leads to the accumulation of extracellular glutamate and subsequently neuronal death. Additionally, astrocytes are responsible for the defense mechanism against toxicants entered into the brain. One of this mechanism is the release of inflammatory cytokines, leading to innate inflammation. However, excessive secretion of proinflammatory cytokines i.e. TNF-α, IL-1s (IL-1α, IL-1β), IL-6 and IL-10 contributed to the chronic inflammation in the CNS, resulting in neuronal injury and later on neurodegenerative diseases [16].

Previous studies of cadmium toxicity on astrocytes focused on oxidative stress and there is little known about cadmium effects on astrocytic inflammation together with its underlying mechanism. Herein, we investigated the effects of cadmium at a toxic concentration on the expression of proinflammatory cytokines (TNF-α, IL-1β and IL-6) and EAAT2 glutamate transporter in human astrocytes. We also showed that NF-κB plays an important role in the up-regulation of cytokines by cadmium in human astrocytes.

Materials and Methods

Cell culture and preparation of cadmium chloride and NF-κB inhibitor

Human astrocytoma U87-MG cell line was obtained from American Type Culture Collection (ATCC). Cells were cultured at 37°C in a humidified chamber with 5% CO_2 in Minimum Essential Medium (MEM) supplemented with 10% fetal bovine serum, 1% sodium pyruvate and 1% penicillin-streptomycin. The completed medium was renewed every 1-2 days.

Cadmium chloride ($CdCl_2$) was dissolved in sterile water at a concentration of 1000 mM and stored at -20°C until use. Working solution of $CdCl_2$ was freshly diluted in MEM plus 1% sodium pyruvate prior to use. SC514 (SML0557, Sigma-Aldrich) was dissolved in dimethyl sulfoxide (DMSO) and diluted with MEM to 20 μM. The final concentration of DMSO is 0.1%.

MTT cell viability assay

U87-MG cells were plated at 1.5×10^4 cells/well in 96-well plates. At 70-80% confluence, cells were treated with 30 μM of $CdCl_2$ for 6 hours and 24 hours in serum-free MEM. At 2 hours before the indicated incubation time, MTT solution was added to each well to a final concentration of 0.5 mg/ml. After that, the MTT solution was discarded carefully and 50 μl DMSO was added to each well to dissolve formazan crystals. The amount of formazan of each well was determined spectrophotometrically by measuring the absorbance at 562 nm in a microplate reader. Each concentration was tested at least 5 replicates. The percentage of cell viability was calculated using the following equation.

$$\%\text{Cell viability} = \frac{\text{Absorbance of treated cells} \times 100}{\text{Absorbance of mock-treated cells}}$$

Quantitative real time PCR

U87-MG cells were plated at 5×10^5 cells in 60-mm dishes. At 70-80% confluence, cells were treated with $CdCl_2$ at 30 μM in serum-free media for 3 hours. Total RNA was extracted and purified by Total RNA Purification kit according to manufacturer's protocol (Jena Bioscience, Germany). Complementary DNA (cDNA) was synthesized using 1 μg of total RNA mixed with random hexamer primers (Roche Diagnostics, USA) and superscript III reverse transcriptase (Invitrogen, USA) at 55ºC for 1 hour. The cDNA product was then amplified for TNF-α, IL-1β, IL-6, EAAT2 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) with the forward primers and reverse primers shown in Table 1. Quantitative Real-time PCR analysis was performed using SensiFAST SYBR LO-ROX (Bioline, Canada) on an Applied Biosystems real time PCR 7500 system. Briefly, the reverse transcribed cDNA was denatured at 95°C for 2 minutes and amplified for 45 cycles (95°C for 15 seconds, 58°C for 30 seconds and 72°C for 30 seconds). The mRNA levels of gene of interest were normalized to the expression of GAPDH. Gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method.

Enzyme-linked immunosorbent assay (ELISA) to measure the release IL-6

U87-MG cells were plated at 1.2×10^5 cells/well in 12-well plates. At 70-80% confluence, cells were pretreated for 1 hour with NF-κB inhibitor, SC514, as previously described and then treated with $CdCl_2$ at 30 μM in serum-free media for 3 hours and 6 hours. At the indicated time, cell culture supernatant from U87-MG cells was collected and centrifuged at 5,000 rpm for 5 minutes. Cell supernatant was collected and stored at -80°C until use. The amount of IL-6 was measured by human IL-6 ELISA kit according to the manufacture protocol (eBioscience, USA).

Statistical Analysis

Each experiment was performed 3 to 4 times and the data was expressed as mean ± SEM. Two-sample comparisons were carried out using Student's t-test. Multiple-sample comparisons were carried out using two-way ANOVA followed by Tukey's pairwise comparison. In all comparisons, differences were considered significant when p value is less than 0.05 ($p \leq 0.05$) and 0.01 ($p \leq 0.01$). All statistical analyses were

performed using Graphpad® Prism statistical analysis software.

Table1: Primer sequences for measuring the mRNA expression.

Target gene	Primer	Sequence
TNF-α	Forward	5′CCCAGGGACCTCTCTCTAATCA3′
	Reverse	5′GCTACAGGCTTGTCACTCGG3′
IL-1β	Forward	5′CCAAAGAAGAAGATGGAAAAGC3′
	Reverse	5′GGTGCTGATGTACCAGTTGGG3′
IL-6	Forward	5′ACCCCTGACCCAACCACAAAT3′
	Reverse	5′AGCTGCGCAGAATGAGATGAG3′
EAAT2	Forward	5′TTCCCTGAAAACCTTGTCCAA3′
	Reverse	5′GGTGGTGCAACCAGGACTTT3′
GAPDH	Forward	5′AGCCTTCTCCATGGTGGTGAAGAC3′
	Reverse	5′CGGAGTCAACGGATTTGGTCG3′

Results

Cadmium decreases cell viability of human astrocytes

We initially determined the cytotoxicity of cadmium on U87-MG astrocytoma cells by MTT assays. 40% astrocytic cell death was observed at 6 hour post-exposure to $CdCl_2$. Cell death continued to increase with the time of cadmium incubation. At 24 hours, cadmium reduced cellular viability of astrocytes to 40%. These results indicated that exposure to 30 μM of $CdCl_2$ was toxic to human astrocytes and cadmium induced astrocyte death in a time dependent manner

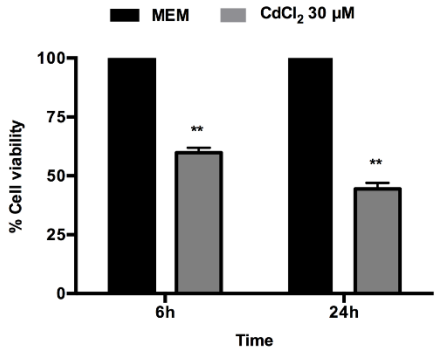


Figure 1. Acute exposure to cadmium was cytotoxic to human astrocytes. U87-MG astrocytoma cells were treated with $CdCl_2$ at 30 μM for 6 hours and 24 hours. Cell viability of astrocytes was analyzed by MTT assays. Each bar represented the percentage of surviving cells (mean ± SEM) of four independent experiments (n=4). Student's t-test was used for statistical analysis and statistical significance was donated as ** ($p \leq 0.01$) compared to mock-tread cells at the same timepoint.

Cadmium induces the expression of proinflammatory cytokines as TNF-α, IL-1β and IL-6, but not EAAT2 in human astrocytes

Next, we studied the effects of cadmium on inflammation and function of astrocytes. Cadmium could stimulate the release of proinflammatory cytokines in other cells such as human type 2 epithelial cells, alveolar macrophage and human lung cells [11,12]. We found that at 3 hour post-exposure to 30 μM $CdCl_2$, levels of TNF-α, IL-1β and IL-6 were significantly increased with 2.92±0.34, 3.62±1.39 and 18.76±4.93 folds, respectively. However, the expression of EAAT2, a common glutamate transporter in adult brain, was not affected. These results suggested that cytotoxicity from acute exposure to cadmium may be a result from the elevation of proinflammatory cytokines. Additionally, decreased glutamate uptake may less likely to contribute to the cell death at early stage of toxicity.

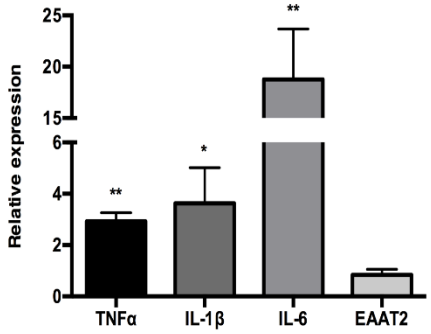


Figure 2. Cadmium increased expression of proinflammatory cytokines including TNF-α, IL-1β and IL-6, but did not alter EAAT2 expression in human astrocytes. Total RNA was extracted from U87-MG cells at 3 hours after exposure to 30 μM $CdCl_2$. Levels of mRNA transcripts of TNF-α, IL-1β, IL-6 and EAAT2

were determined using real time PCR. The expression of each gene was normalized to housekeeping gene GAPDH and presented as relative expression to mock-treated cells. Each bar represented mean values (± SEM) of three independent experiments (n=3). Student's t-test was used for statistical analysis and statistical significance was donated as * ($p \leq 0.05$) and ** ($p \leq 0.01$) compared to mock-tread cells.

Cadmium induces the secretion of IL-6 by human astrocytes

Because the up-regulation of IL-6 mRNA expression is higher than TNF-α and IL-1β, we further confirmed whether the increased transcription levels of IL-6 by cadmium was reflected in the increase of this cytokine secretion using ELISA assays. There was significant increase of IL-6 (pg/ml) presented in the culture medium at 3 hours after exposure to 30 μM $CdCl_2$ as compared to mock-treated cells (2.69±1.62 vs 34.04±7.30). The levels of IL-6 secretion from U87-MG cells continued to increase with time to 84.85±7.99 at 6 hours. Our results indicated that cadmium induces the increase of IL-6 secretion from astrocytes.

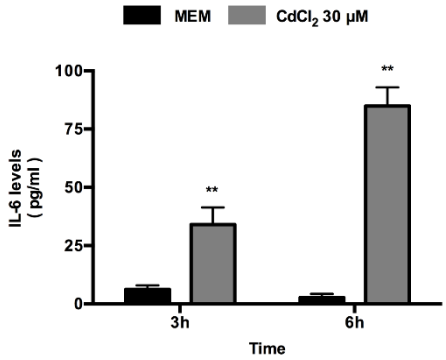


Figure 3. Cadmium induced IL-6 release from human astrocytes. Cell culture supernatant was collected at 3 hour and 6 hour post-exposure to $CdCl_2$ (30 μM). IL-6 protein was measured by human IL-6 ELISA kit according to the manufacture protocol and shown as pg/ml of culture supernatant. Each bar represented mean value (±SEM) of three independent experiments (n=3). Student's t-test was used for statistical analysis and statistical significance was donated as ** ($p \leq 0.01$) compared to mock treated cells at the same time point.

Cadmium-induced IL-6 expression by human astrocytes is mediated by the NF-κB pathway

NF-κB is a main pathway that controls secretion of many inflammatory cytokines. Cadmium was previously reported to activate NF-κB pathway and resulting in the increased expression and secretion of IL-6 in THP-1 human monocytic leukemia cells [13] and human lung epithelial cells [14]. In order to determine the role of this pathway in cadmium-induced secretion of IL-6, we used SC514 to block NF-κB activation. SC514 is an IκB kinase 2 (IKK-2) inhibitor, which blocks IκB phosphorylation and degradation, leading to the inhibition of NF-κB activation. The dose at 20 μM SC514 was selected based on the study in Tat-infected human astrocyte cells (SVGA cells) [17]. Human astrocytes were pretreated with NF-κB inhibitor (20 μM) for 1 hour followed by $CdCl_2$ 30 μM for 6 hours based on our result in Fig. 3. Our result showed that SC514 decreased the cadmium-induced secretion of IL-6 from human astrocytes by approximately 70% compared with cadmium-treated cells (25.30 ±2.53 vs 84.85±7.99). This result suggested that cadmium induces the secretion of IL-6 from astrocytes via the NF-κB dependent pathway.

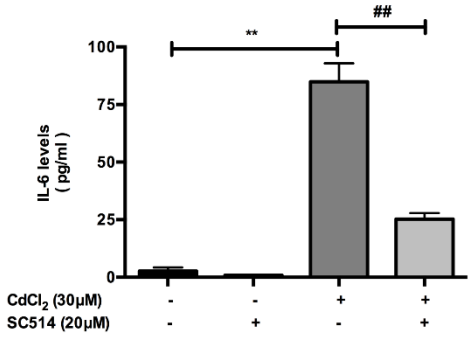


Figure 4. An NF-κB inhibitor inhibited the excessive production of IL-6 induced by cadmium in human astrocytes. Cell culture supernatant was collected at 6 hours after exposure human astrocytes to $CdCl_2$ (30 μM) with or without 20 μM SC514, an NF-κB inhibitor. IL-6 protein was measured by human IL-6 ELISA kit according to the manufacture protocol and shown as pg/ml of culture supernatant. Each bar represented mean value (±SEM) of three independent experiments (n=3). Two-way ANOVA was used for statistical analysis. Statistical significance was donated as ** ($p \leq 0.01$) in the comparison between cadmium-treated cells and mock-treated cells and ## ($p \leq 0.01$) in the comparison between cadmium-treated cells and cadmium-treated cells with an NF-κB inhibitor.

Discussion

Two major findings of this study are the cytotoxicity of cadmium to human astrocytes and the promotion of proinflammatory cytokines by cadmium. In term of cytotoxicity, our results showed that the astrocytes might be more resistance to cadmium toxicity than neurons. We reported here that human astrocytes exposed to 30 μM of $CdCl_2$ up to 24 hours showed a 40% reduction in cell viability. While another study on

primary rat neurons at the same duration reported that exposure to only 5 μM of $CdCl_2$ reduced cell viability of neurons to 50% [18].

Most of previous studies on cadmium toxicity to the central nervous system were focused on oxidative stress; however, cadmium could induce inflammatory response by promoting the release of proinflammatory cytokines. Cadmium significantly induced IL-1β, TNF-α, MIP-2 and IL-6 secretion from human type 2 epithelial cells, alveolar macrophage [11] and human lung cells [12]. Similar to these experiments, we found that toxic concentration of cadmium strongly enhanced the expression of TNF-α, IL-1β, and IL-6 in human astrocytes. Our results also showed that the increased levels of IL-6 mRNA were associated with the increase of this cytokine secretion, suggesting that cadmium exposure could promote the release of proinflammatory cytokines from astrocytes and subsequently cause neuroinflammation. Elevation of TNF-α and IL-6 level has been detected in plasma of rats after exposed to intraperitoneal injection of 2.5 mg/kg cadmium [19] and in liver tissues of mice exposed to 40 mg/L cadmium in drinking water [20]. The up-regulation of TNF-α and IL-1β were less than the increase of IL-6. The study of Souza et al., using human hepatocellular carcinoma cells, indicated that the expression of IL-1β mRNA peaked at 1 hour after exposure to cadmium [21]. Thus, it is possible that at 3 hour post-exposure in our experiment, the peak of IL-1β mRNA expression was already passed.

Astrocytes are one of the first brain cells to expose to any toxicant that crosses blood brain barrier. A recent study by Ciesielski et al. indicated that there was a significant association between children with high cadmium level in urine and learning disability [4]. Elevated serum levels of IL-1s (IL-1α, IL-1β), TNF-α, IL-6 and IL-10 have been found in patients with Alzheimer's disease, mild cognitive impairment and Parkinson's disease [16]. Therefore, it is possible that inflammation induced by cadmium may contribute to cytotoxicity and the pathogenesis of neurocognitive impairment.

Astrocytes are also known to be responsible for glutamate uptake through glial glutamate transporters (i.e. GLAST/EAAT1, EAAT2/GLT-1). Dysfunction of astrocytic glutamate transporters induced by toxicants and infection leads to the accumulation of extracellular glutamate, neuronal excitotoxicity and CNS disorders such as amyotrophic lateral sclerosis (ALS) and epilepsy [22]. In this study, we demonstrated that the acute exposure of cadmium to human astrocytes had no effect on the expression of EAAT2 at 3 hour post-exposure. On the other hand, Liu et al. reported that exposure of primary neonatal rat astrocytes to a non-toxic dose of cadmium inhibited astrocytic glutamate uptake activity by reducing the transcription of glutamate aspartate transporter (GLAST/EAAT1) gene at 24 hours [10]. Expression of GLAST showed mainly in developmental brain, whereas EAAT2 expression mostly observed in adult brain [23]. Thus, it is possible that the effect of cadmium on the disruption of glutamate uptake in astrocytes may depend on cell types, concentration of cadmium and exposure time.

NF-κB is a major transcription factor, which is responsible for the regulation of many cytokines and chemokines including TNF-α, IL-1β and IL-6. Previous studies showed that cadmium exposure could elevate the binding and activation of NF-κB and subsequently increase mRNA and protein levels of these proinflammatory mediators in THP-1 human monocytic leukemia cells [13] and human lung epithelial cells [14]. The release of TNF-α induced by cadmium through NF-κB activation resulted in the increased expression of cadmium transporter ZIP8, leading to higher uptake of cadmium into human lung epithelial cells and subsequently cell death [14]. Similarly, we found that inhibition of NF-κB activation decreased the release of IL-6. Although we did not test the effect of NF-κB activation on the up-regulation of TNF-α and IL-1β, we speculated that inhibition of NF-κB will decrease the expression of these cytokines. Additionally, these cytokines may be responsible for cytotoxicity effect of cadmium on astrocytes similar to its toxicity on lung epithelial cells.

Conclusions

Our results suggested that in addition to the direct toxicity, cadmium also could promote inflammatory response in the brain. At 3 hour post-exposure, cadmium could up-regulate the expression of proinflammatory cytokines such as TNF-α, IL-1β and IL-6 in human astrocytes by activating the NF-κB pathway. Therefore, the inhibition of NF-κB pathway could be the potential therapeutic target for prevention of cadmium-induced neuroinflammation and toxicity in the CNS.

Acknowledgements

We thank Ms. Pannaree Piromkraipak, Mr. Piyadon Sathavorasmith, and Mr. Kant Sangpairoj for their technical assistance. We thank central instrument facility (CIF) for using their equipment. This study was supported by the development and promotion of science and technology talented project to SP.

References

[1] Mendez-Armenta M, Rios C, (2007). Cadmium neurotoxicity. Environ Toxicol Pharmacol.

23(3):350-8.
[2] Wang B, Du Y, (2003). Cadmium and its neurotoxic effects. Oxid Med Cell Longev. 2013:898034.
[3] Al-Saleh I, Shinwari N, (2001). Levels of cadmium, lead, and mercury in human brain tumors. Biol Trace Elem Res. 79(3):197-203.
[4] Ciesielski T, Weuve J, Bellinger DC, Schwartz J, Lanphear B, Wright RO, (2012). Cadmium exposure and neurodevelopmental outcomes in U.S. children. Environ Health Perspect. 120(5):758-63.
[5] Okuda B, Iwamoto Y, Tachibana H, Sugita M, (1997). Parkinsonism after acute cadmium poisoning. Clin Neurol Neurosurg. 99(4):263-5.
[6] Du Y, Shang Q, (2006). [Review of effect on human health for environmental cadmium pollution]. Wei Sheng Yan Jiu. 35(2):241-3.
[7] Lopez E, Arce C, Oset-Gasque MJ, Canadas S, Gonzalez MP, (2006). Cadmium induces reactive oxygen species generation and lipid peroxidation in cortical neurons in culture. Free Radic Biol Med. 40(6):940-51.
[8] Hossain S, Liu HN, Nguyen M, Shore G, Almazan G, (2009). Cadmium exposure induces mitochondria-dependent apoptosis in oligodendrocytes. Neurotoxicology. 30(4):544-54.
[9] He W, Li Y, Tian J, Jiang N, Du B, Peng Y, (2015). Optimized mixture of As, Cd and Pb induce mitochondria-mediated apoptosis in C6-glioma via astroglial activation, inflammation and P38-MAPK. Am J Cancer Res. 5(8):2396-408.
[10] Liu YP, Yang CS, Tzeng SF, (2008). Inhibitory regulation of glutamate aspartate transporter (GLAST) expression in astrocytes by cadmium-induced calcium influx. J Neurochem. 105(1):137-50.
[11] Lag M, Rodionov D, Ovrevik J, Bakke O, Schwarze PE, Refsnes M, (2010). Cadmium-induced inflammatory responses in cells relevant for lung toxicity: Expression and release of cytokines in fibroblasts, epithelial cells and macrophages. Toxicol Lett. 193(3):252-60.
[12] Odewumi C, Latinwo LM, Sinclair A, Badisa VL, Abdullah A, Badisa RB, (2015). Effect of cadmium on the expression levels of interleukin-1alpha and interleukin-10 cytokines in human lung cells. Mol Med Rep. 12(5):6422-6.
[13] Freitas M, Fernandes E, (2011). Zinc, cadmium and nickel increase the activation of NF-kappaB and the release of cytokines from THP-1 monocytic cells. Metallomics. 3(11):1238-43.
[14] Napolitano JR, Liu MJ, Bao S, Crawford M, Nana-Sinkam P, Cormet-Boyaka E, et al., (2012). Cadmium-mediated toxicity of lung epithelia is enhanced through NF-kappaB-mediated transcriptional activation of the human zinc transporter ZIP8. Am J Physiol Lung Cell Mol Physiol. 302(9):L909-18.
[15] Cormet-Boyaka E, Jolivette K, Bonnegarde-Bernard A, Rennolds J, Hassan F, Mehta P, et al., (2012). An NF-kappaB-independent and Erk1/2-dependent mechanism controls CXCL8/IL-8 responses of airway epithelial cells to cadmium. Toxicol Sci. 125(2):418-29.
[16] Dursun E, Gezen-Ak D, Hanagasi H, Bilgic B, Lohmann E, Ertan S, et al., (2015). The interleukin 1 alpha, interleukin 1 beta, interleukin 6 and alpha-2-macroglobulin serum levels in patients with early or late onset Alzheimer's disease, mild cognitive impairment or Parkinson's disease. J Neuroimmunol. 283:50-7.
[17] Liu X, Kumar A, (2015). Differential signaling mechanism for HIV-1 Nef-mediated production of IL-6 and IL-8 in human astrocytes. Sci Rep. 5:9867.
[18] Yuan L, Liu A, Qiao L, Sheng B, Xu M, Li W, et al., (2015). The relationship of CSF and plasma cytokine levels in HIV infected patients with neurocognitive impairment. Biomed Res Int. 2015:506872.
[19] Lee J, Lim KT, (2011). Preventive effect of phytoglycoprotein (27 kDa) on inflammatory factors at liver injury in cadmium chloride-exposed ICR mice. J Cell Biochem. 112(2):694-703.
[20] Alghasham A, Salem TA, Meki AR, (2013). Effect of cadmium-polluted water on plasma levels of tumor necrosis factor-alpha, interleukin-6 and oxidative status biomarkers in rats: protective effect of curcumin. Food Chem Toxicol. 59:160-4.
[21] Souza V, Escobar Md Mdel C, Gomez-Quiroz L, Bucio L, Hernandez E, Cossio EC, et al., (2004). Acute cadmium exposure enhances AP-1 DNA binding and induces cytokines expression and heat shock protein 70 in HepG2 cells. Toxicology. 197(3):213-28.
[22] Bar-Sela S, Reingold S, Richter ED, (2001). Amyotrophic lateral sclerosis in a battery-factory worker exposed to cadmium. Int J Occup Environ Health. 7(2):109-12.
[23] Swanson RA, Liu J, Miller JW, Rothstein JD, Farrell K, Stein BA, et al., (1997). Neuronal regulation of glutamate transporter subtype expression in astrocytes. J Neurosci. 17(3):932-40.
ERK1/2 MAPK in human airway epithelial cells [15].

A Double Blind Randomized Controlled Clinical Trial of Regro Genesis Serum Versus 5% Topical Minoxidil in the Treadment of Androgenetic Alopecia in Thai Men

Chindanai Vigraipat[1*]

[1]*Master of Science Program in Dermatology, School of Anti-Aging and Regenerative Medicine, Mae Fah Luang University, Asoke road Sukhumvit21 Wattana Bangkok 10110 Thailand*

[*]*E-mail: 5752001004@lamduan.mfu.ac.th*

Abstract

Nowadays androgenetic alopecia (AGA) is a major concern problem in population. 5% topical minoxidil is one of standard pharmaceutical treatment approved by the US FDA. However the side effects were local irritation, allergic contact dermatitis, and hypertrichosis. Saw palmetto and procapil have research that they are effective on treatment of AGA. In this study, we compare the efficacy of Regro Genesis Serum that contained saw palmetto and procapil to 5% topical minoxidil for treatment of AGA in Thai men. The aim of study is to determine the efficacy of Regro Genesis Serum compared to 5% topical minoxidil for hair growth. Twenty male subjects (aged 20- 60 years old) with AGA (Hamiton-Norwood classification II-IIIv) were enrolled. Study design was double-blind, randomized, controled clinical trial. Evaluation was performed every 4-week interval using hair diameters and hair counts measured by Folliscope, photographs from VISIA® and records of side effects. Volunteers' satisfaction was assessed by questionnaires at 12th week. Results, eighteen volunteers completed the study. Both sides of head had statistically significant increase from baseline in global photographic score (mean global photographic score before treatment is 0 and after treatment until week 12 is 2.44 in side of treatment by Regro Genesis Serum, mean global photographic score before treatment is 0 and after treatment until week 12 is 2.31 in side of treatment by 5% topical minoxidil). Means of hair numbers and hair diameters had statistically significant increase from baseline both sides score (means of hair numbers and hair diameters before treatment is 18.50/0.5cm^2 and 30.44 micrometers and after treatment until week 12 is 37.56/0.5 cm^2 and 57.25 micrometers in side of treatment by Regro Genesis Serum, means of hair numbers and hair diameters before treatment is 18.88/0.5cm^2 and 30.56 micrometers and after treatment until week 12 is 37.75/0.5 cm^2 and 57.25 micrometers in side of treatment by 5% topical minoxidil). Few participants had mild pruritus and mild local irritation in both groups. Conclusion, Regro Genesis Serum is equally effective on hair growth to 5% topical minoxidil. And also safe with few local side effects.

Key words: Regro Genesis Serum/Topical minoxidil/Hair

Introduction

Hair and hair styles can help human attractive. The thickness and healthy of human hair are the desirable of individual. Nowadays AGA is major concern problem in population. Treatment of AGA is currently more interest. [1]

In the treatment of AGA, current drug approved by The Food and Drug Administration of the United state (FDA) and Thailand for hair growth is topical minoxidil. It is approved for the treatment of Androgenetic alopecia (AGA). ([2] In term of side effect, topical minoxidil is also common such as local irritation, allergic contact dermatitis, erythema at site of application, and hypertrichosis. [3]

Regro Genesis Serum contained of saw palmetto and procapil. Saw palmetto and procapil have research that they are effective for treatment of AGA. Saw palmetto inhibited enzyme 5α-reductase that inhibited hormone testosterone to be dihydrotestosterone. Procapil contained of biotinyl-GHK, apigenine, and olenolic acid can inhibited enzyme 5 α-reductase, promoted blood flow to hair root, and promoted hair follicle keratinocytes. Procapil had research from institute of dermatology that they have statistically significant improve in hair numbers and hair diameters when follow up to 16 weeks. Saw palmetto and procapil can prevent hair loss and promote hair growth. [4]

This study is to determine the efficacy of Regro Genesis Serum compared to 5% topical minoxidil for

hair growth. Global photographic score, hair diameters and hair numbers are evaluated in this study.

Objective

The aim of study is to determine the efficacy of Regro Genesis Serum compared to 5% topical minoxidil for hair growth.

Material and Methods

Twenty male subjects (aged 20-60 years old) with AGA (Hamiton-Norwood classification II-IIIv) were enrolled. Study design was double-blind, randomized, controled clinical trial. Randomly selected for applied either Regro Genesis Serum and 5% topical minoxidil on right and left sides of head. Evaluation was performed every 4-week interval using hair diameters and hair counts measured by Folliscope, photographs from VISIA® and records of side effects. Volunteers' satisfaction was assessed by questionnaires at 12th week.

Statistical analysis

Global photographic scores were calculated by Mann-Whitney U test. Mean changes of hair diameters and hair numbers in same side before and after applying drug were calculated by Pair T-test. Mean changes of hair diameters and hair numbers to other side before and after applying drug were calculated by Unpaired t-test. Satisfaction score and side effects were calculated by Fisher-Exact test.

Results

Eighteen volunteers completed the study. All of the subjects are Thai men with AGA (Hamiton-Norwood classification II-IIIv). The average participant age was 33.31 ± 7.61 years old. Both sides of head had statistically significant increase from baseline in global photographic score (mean global photographic score before treatment is 0 and after treatment until week 12 is 2.44 ± 0.51 in side of treatment by Regro Genesis Serum, mean global photographic score before treatment is 0 and after treatment until week 12 is 2.31 ± 0.48 in side of treatment by 5% topical minoxidil). Analyzing in group showed Regro Genesis Serum statistically significant in global photographic score since 4th week (p=0.009) and 5% topical minoxidil statistically significant in global photographic score since 4th week (p=0.04).(Figure1)

When compared the efficacy between Regro Genesis Serum and 5% topical minoxidil for hair growth found that Global photographic scores in Regro Genesis Serum group was not significantly different to 5% topical minoxidil group after 4th, 8th, and 12th weeks. (p = 0.462, p = 0.462, and p = 0.481) (Figure2)

Means of hair numbers and hair diameters had statistically significant increase from baseline both sides of head (means of hair numbers and hair diameters before treatment is 18.50 ± 3.23/0.5cm^2 and 30.44 ± 4.23 micrometers and after treatment until week 12 is 37.56 ± 3.56/0.5 cm^2 and 57.25 ± 3.94 micrometers in side of treatment by Regro Genesis Serum, means of hair numbers and hair diameters before treatment is 18.88 ± 2.73/0.5cm^2 and 30.56 ± 5.73 micrometers and after treatment until week 12 is 37.75 ± 3.28/0.5 cm^2 and 57.25 ± 3.61 micrometers in side of treatment by 5% topical minoxidil). Analyzing in group showed both Regro Genesis Serum and 5% topical minoxidil statistically significant in hair numbers and hair diameters since 4th week (p<0.001, p<0.001). (Figure3) (Figure5)

When compared both groups, hair numbers and hair diameters in Regro Genesis Serum group was not significantly different to 5% topical minoxidil after 4th, 8th, and 12th weeks. (p = 0.620, 0.487, p = 0.751, 0.719, and p = 0.878, 1.0). (Figure4) (Figure6)

Additionally satisfaction score and side effects in Regro Genesis Serum group were not significantly different to 5% topical minoxidil group after 12th weeks. (p = 0.43, p = 0.30). Side effects were occurred mild pruritus and mild local irritation.

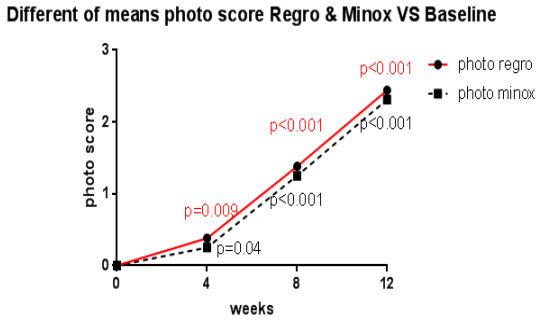


Figure 1 Linear graphs showed different of means Global photographic score Regro Genesis Serum & 5% topical minoxidil VS Baseline before treatment.

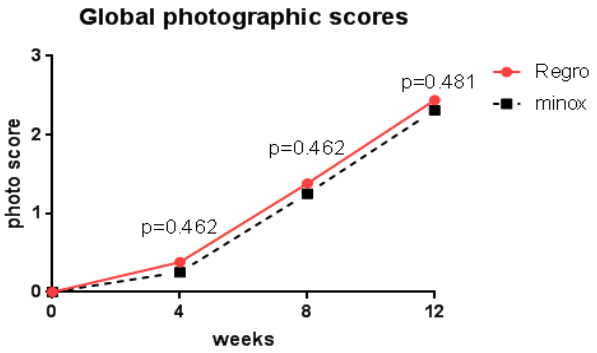


Figure 2 Linear graphs showed comparison Global photographic score between Regro Genesis Serum and 5% topical minoxidil.

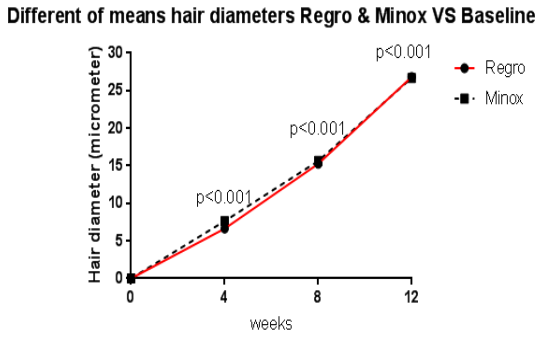


Figure 3 Linear graphs showed different of means hair diameters Regro Genesis Serum & 5% topical minoxidil VS Baseline before treatment.

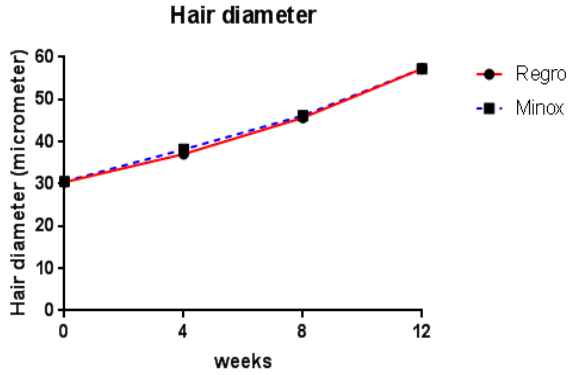


Figure 4 Linear graphs showed comparison Hair diameters between Regro Genesis Serum and 5% topical minoxidil.

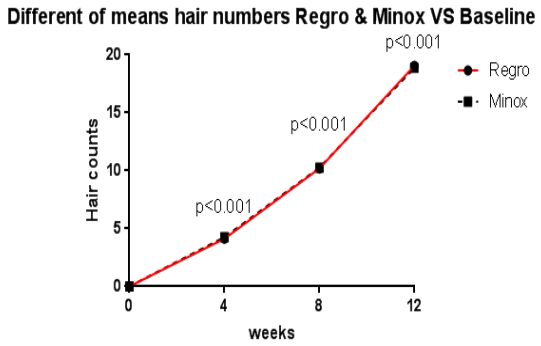


Figure 5 Linear graphs showed different of means hair diameters Regro Genesis Serum & 5% topical minoxidil VS Baseline before treatment.

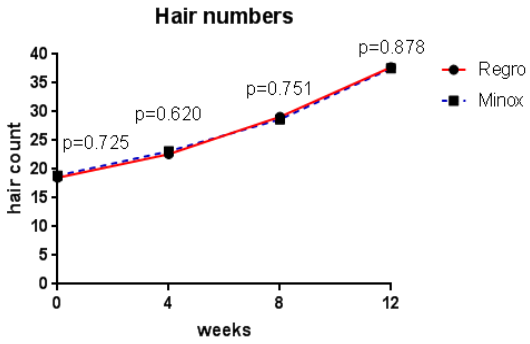


Figure 6 Linear graphs showed comparison Hair numbers between Regro Genesis Serum and 5% topical minoxidil.

Discussion

From this study, we evaluated that the efficacy of Regro Genesis Serum was statistically significant effective on hair growth since 4th week from baseline. This possible explained by Regro Genesis Serum contained of saw palmetto and procapil. Saw palmetto inhibited enzyme 5α-reductase that inhibited hormone testosterone to be dihydrotestosterone. Procapil inhibited enzyme 5 α-reductase, promoted blood flow to hair root, and promoted hair follicle keratinocytes.(Puglin, Chanon, and Thanonggiart, 2553). When compared with 5% topical minoxidil (one of standard treatments of AGA), the efficacy of Regro Genesis Serum was not significantly different to 5% topical minoxidil for hair growth at 4th, 8th, and 12th weeks.

The research outcomes were evaluated by visual evaluation. We used VISIA®, which compared photos in the same positioning and standardized lighting. Our findings, global photographic scores showed no significantly different between both groups. About hair diameters and hair numbers, we were evaluated by folliscope. Our findings, hair diameters and hair numbers showed no significantly different between both groups too. This may be explaned that Regro Genesis Serum was equally effective on hair growth to 5% topical minoxidil. Volunteers' satisfaction scores rated Regro Genesis Serum more than 5% topical minoxidil. However, no statistically difference of volunteers' satisfaction scores ($p = 0.43$).

A few subjects also reported mild local irritation and pruritus in both groups and completely cured. Two volunteers discontinued from this study because of transportation.

Conclusion

In Thai men, Regro Genesis Serum is equally effective on hair growth to 5% topical minoxidil group. And also safe with few local side effects.

Suggestion

The effectiveness on hair growth of Regro Genesis Serum should be performed in long term for more information about efficacy and side effects.

Additionally, the study should be performed after drug withdrawal for more information about hair loss.

References

สุนิสา ไทยจินดา, (2551). ผมร่วง และศรีษะล้าน, *สงขลานครินทร์เวชสาร, 26*(6), 587-599.

Rogers, E.N. & Avram, R.M. (2008). Medical treatments for male and female pattern hair loss, *J Am Acad Dermatol, 59*, 547-566.

Guarneri, C. & Cannavò, S.P. (2013), Erosive pustular dermatosis of scalp from topical minoxidil 5% solution, *Int J Dermatol*,*52*(4), 507-509.

พู่กลิ่น ตรีสุโกศล, ชนนท์ ธรรมวิจิตรกุล และทะนงเกียรติ เทียนถาวร. (2553). ประสิทธิภาพการรักษาด้วย Intensive serum hair treatment (Regro®) ในผู้ป่วยศีรษะล้าน: 4-months randomized double-blind placebo controlled clinical trial, รวมบทคัดย่อการประชุมใหญ่สามัญประจำปี 2553. วารสารโรคผิวหนัง สถาบันโรคผิวหนัง กรมการแพทย์ กระทรวงสาธารณสุข, 2553(1)

A Double-Blind Randomized Controlled Trial of the Efficacy of Centella Asiatica Extract Cream Compare with Standard Cream Base for Facial Shin Whitening

Patsuda Chomchan[1] and Assoc. Prof. Sunisa Thaichinda[2*]

[1]Student in program Master of Science,[2]Corresponding author
Master of Science Program in Dermatology, School of Anti-Aging and Regenerative Medicine, Mae Fah Luang University, Thailand
**Corresponding author. Tel.:+6626644361, E-mail: dr.sunisa.t@gmail.com*

Abstract

Centella Asiatica is a Thai folk herb known as Bai bua bok with many widely known properties. Recently, there have been additional researches conducted on Centella Asiatica extracts. It has dermatological benefits including collagen production, reduction in collagen degradation , promote wound healing and scar treatment. Previous studies have demonstrated that Centella Asiatica extracts can also inhibit tyrosinase enzyme and antioxidant effects. The purpose of this study is to compare the efficacies of Centella Asiatica cream with standard cream base in facial skin whitening.
Objective: To compare efficacy of Centella Asiatica Cream and standard cream base in facial skin whitening
Material and Methods: Thirty Thai volunteers were enrolled to this randomized, split face comparative study. Centella Asiatica cream and standard cream base were randomly applied in the split face design (right and left sides) twice daily for 12 weeks with follow up every 4 weeks. Skin whitening was evaluated by using mean melanin index measured by Mexameter MX18 at week 4, 8 and 12. Photographs from VISIA® Complexion Analysis System at week 0 (before treatment) and at week 12 were compared and scored evaluation scales by three doctors. Volunteers' satisfaction were evaluated at week 12 through questionnaires. Volunteers' side effects were assessed through questionnaires and physician observation.
Results: Thirty subjects completed the study. The subjects were female (22/30, 73.3%) and male (8/30, 26.7%). The average participant age was 31.23 ± 4.89 years old. The subject were skin type 3 (22/30,73.3%) and skin type 4 (8/30, 26.7%). The results revealed mean melanin index of Centella Asiatica cream was significantly superior to the standard cream base from week 4 until week 12. In addition, the participants were significantly more satisfied with Centella Asiatica cream than standard cream base. No adverse reaction was found
Conclusion: Centella Asiatica cream could accomplish significantly better outcomes in all parameters compared to standard cream base from 4th week of treatment onwards with no recorded side effects. Therefore, suggesting that Centella Asiatica cream is effective for facial whitening.

Keywords: Centella Asiatica/Standard cream base/Topical/ Whitening

Introduction

Having fair skin is currently a prominent beauty trend in Asian countries including Thailand. While many whitening products are being widely used[1], the whitening and bleaching agents launched into the market can be identified as either synthetic or organic. While certain substances carry serious side effects such as hydroquinone, safer products are more expensive.

Skin color is the result of the spectrum of light and its absorption and reflection by chromophores in the skin, determined by melanin amount, type, and intracellular distribution and location within the skin.[2]Moreover, there are other factors that determine the skin color such as genetics, hormones, ultraviolet and certain drugs.[3]

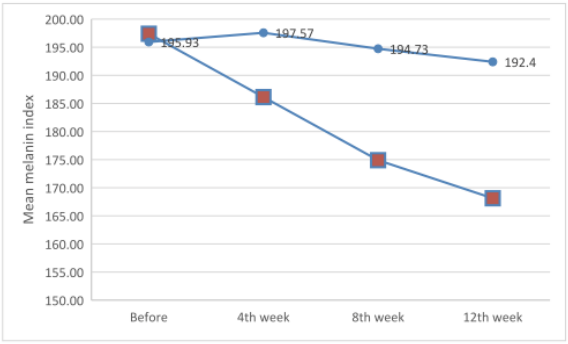
Whitening agent has many mechanisms to decrease melanin in the skin but almost all of whitening agents act as tyrosinase-enzyme inhibitors, which regulate a rate-limiting step of melanin synthesis such as Hydroquinone, Aloesin, Arbutin, Kojic acid and Flavonoid.[4]

In general, consumers are more focused on natural products due to the widespread belief that they cause less side effects and are more suitable for longterm use. This in turn, has lead to the increasing popularity of organic whitening products.[5]

Centella Asiatica is a Thai folk herb with many widely known properties. Triterpenoid, saponins, the primary constituents of Centella Asiatica are manly believed to be responsible for its wide therapeutic actions. The herb is recommended for the treatment of various skin conditions such as leprosy, lupus, varicose

ulcers, eczema, psoriasis, diarrhea, fever, amenorrhea, diseases of the female genitourinary tract and also for relieving anxiety and improving cognition.[6,7] Recently, there have been additional researches conducted on Centella Asiatica extracts. Which found many dermatological benefits including collagen production and reduction in collagen degradation, so as to promote wound healing and scar treatment.[8] Moreover, the most recent studies have demonstrated that Centella Asiatica extracts can also inhibit tyrosinase enzyme,the key enzyme in melanogenesis.[9] So Centella Asiatica may have efficacy and safety in Facial Skin Whitening

.



Picture1 Linear graphs show mean melanin index of Centella Asiatica side at before, 4th,8th and 12th

Objective

The purpose of this study is to compare efficacy of Centella Asiatica Cream and standard cream base in facial skin whitening

Research methodology

Thirty participants (men and women, aged 25-45 years old) with skin type III to IV were enrolled. After obtaining their consent, Centella Asiatica cream and standard cream base (of similar consistency and color) were applied randomly in the split face design (right and left sides) twice daily for 12 weeks, with follow-up interval of every 4 weeks. Skin whitening was evaluated by using mean melanin index measured by Mexameter MX18 at week 4, 8 and 12. The area of measurement is the area just below midpupillary line (5 centimeters).

Skin whitening evaluation was carried out by comparing means of melanin index measured by Mexameter MX18 at week 4, 8 and 12. Photographs from VISIA® Complexion Analysis System at week 0 (before treatment) and at week 12 were compared and scored evaluation scales by three doctors. Volunteers' satisfaction were evaluated at week 12 through questionnaires. Volunteers' side effects were assessed through questionnaires and physician observation.

Statistical Analysis

Volunteers' research profile data used descriptive statistical analysis to provide descriptive information. Comparison of mean melanin indices from pre-treatment to post-treatment of Centella Asiatica cream and standard cream base were carried out by paired t-test statistics. Comparison of Global photographic score (evaluate by 3 independent doctors) by paired difference between both sides used unpaired t-test statistics. Comparison of mean of Patient satisfaction score of Centella Asiatica cream to standard cream base employed paired t-test statistics, with significance level of p-value <0.05. Assessment of side effects and complications were carried out by descriptive statistical analysis. Summative evaluation use descriptive statistical analysis.

Results

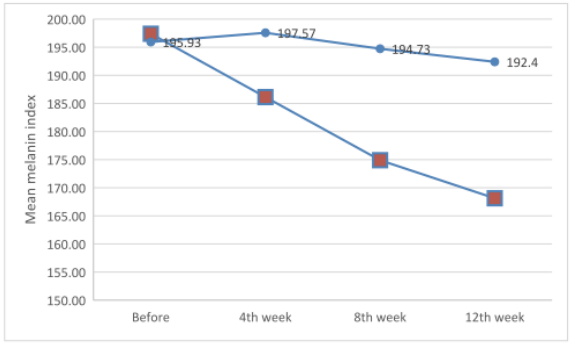
At the end of the study, thirty subjects completed the study. The subjects were female (22/30, 73.3%) and male (8/30, 26.7%). The average participant age was 31.23 ± 4.89 years old. The subject were skin type 3 (22/30,73.3%) and skin type 4 (8/30, 26.7%). Duration that subject exposed to the sun light for 3.33±1.49 hours per day in average.

The results revealed that Centella Asiatica cream showed statistically significant reduction of baseline in mean melanin index ($p < 0.05$) and standard cream base were not statistically significant reduction from the baseline. Also, there were statistically significant reduction of mean melanin indices when compared with the standard cream base from the 4th week of study till the final week (12th week) (picture 1-2).

In addition, the doctor's evaluation and patient satisfaction were performed by questionnaires, the result showed more significant satisfaction with Centella Asiatica cream than standard cream base (picture3) No adverse reaction was found on any participants throughout the 12 weeks study.

Discussion

The results have shown a continuous tendency towards significantly mean improvement of mean melanin index in the Centella Asiatic cream compared with standard cream base from the 4th week of study till the final week. This is consistent with findings in previous studies where Madecassoside (MA), a pentacyclic triterpene isolated from Centella Asitica



Significantly inhibits UV-induced melanin synthesis and melanosome transfer in a keratinocytes and melanocytes. So Madecassoside may be an effective inhibitor of hyperpigmentation caused by UV irradiation.[10]To eliminate affecting external factors, this study was designated as split-face clinical trial, so the divergence of general characteristics of volunteers was insignificant.

Regarding the results of global photographic score by doctors at 12th week, Centella Asiatica cream was more satisfying than standard cream base. This was concordant with volunteers' satisfaction. It is possible that Centella Asiatica cream can improve clinical pictures and give better result for facial whitening.

Concerning side effects, no adverse reaction were observed throughout the 12 weeks study. Thus, the Centella Asiatica extract appeared to be well tolerated and safe, however the extract should be further evaluated for a more prolonged usage.

Before After 12 week
Centella Asiatica

Conclusion

The results of this study clearly demonstrated that Centella Asiatica was able to reduce melanin production in human volunteers. With significant lightening effect, from 4th week of treatment onwards, good satisfaction on the clinical evaluation, volunteers satisfaction and no side effects were observed. Suggesting that Centella Asiatica extract, may have a very promising potential for use as a safe, effective and economical whitening agent.

Suggestion

We suggest further investigations to study the highest concentration with lowest side effects, the duration that Centella Asiatica extract cream will reach its maximum lightening effect and more prolonged usage complication should be find out

References

1. Areeporn Ruksawong. (2011). Behavior of purchasing whitening cream for maintaining face skin of women in Bangkok area. Independent Study of Business Administration. Rajamangala University of Technology Thanyaburi, PathumThani.
2. Kavitha K.Reddy, Yolanda M. Lenzy, Katherine L. Brown and Barbara A. Gilchrest. 2012). Racial consideration:Skin of color. In L. A. Goldsmith, S. I. Katz, B. A. Gilchrest, A. S. Paller, D. J. Leffell & K. Wolff (Eds.), Fitzpatrick's dermatology in general medicine (8th ed., pp. 765-780). New York: McGraw-Hill.
3. Bose SK and Ortonne jp. Pigmentation. In: Textbook of cosmetic dermatology, 2nd ed (Robert B, Howard IM, eds). United Kingdom: Martin Dunitz 1998: 391-416.
4. Petit, L. L. and Piérard, G. E. (2003). Skin-lightening products revisited. International Journal of Cosmetic Science, 25(4), 169.
5.Lin, J., Chiang, H., Lin, Y. and Wen, K. (2008). Natural products with skin-whitening effects. Journal of Food and Drug Analysis, 16(2), 1-10.
6.อัญชลี จูฑะพุทธ. บัวบก:หลักฐานทางวิทยาศาสตร์ที่สนับสนุนการใช้. สถาบันการแพทย์แผนไทย. สืบค้นเมื่อ 5 กันยายน 2558,
7. Gohil KJ, Patel JA and Gajjar AK. (2010). Pharmacological Review on Centella asiatica: A Potential Herbal Cure-all, Indian J Pharm Sci, 72(5), 546-556.
8. นายแพทย์วรวุฒิ เจริญศิริ. คุณค่าใบบัวบกกับผิวสวยใส ในแถบประเทศอาเซียน สืบค้นเมื่อ 5 กันยายน 2558, จาก http://www.phanom.ac.th/musiumphanom/read/page5.html
9. ASTVผู้จัดการออนไลน์. (2558,23 กันยายน). มจธ.วิจัยพบ"ใบบัวบก"มี สารยับยั้งเอนไซม์ไทโรซิเนสสูง เหมาะส่วนผสมเครื่องสำอาง. สืบค้นเมื่อ 24 กันยายน 2558,

จาก

http://www.manager.co.th/iBizChannel/ViewNews.aspx?NewsID=9580000107390

10.Jung E, Lee JA, Shin S, Roh KB, Kim JH and Park D. (2013). Madecassoside inhibits melaninsynthesis by blocking ultraviolet-induced inflammation. Molecules, 18(12), 15724- 15736.

A Double-Blind Randomized Controlled Trial of the Efficacy of Riceberry Extract Cream Compare with 2% Hydroquinone Cream for Melisma

Vatchapon Tanamittramanee[1] and Paisal Rummaneethron[2]

[1]Student in Master of science in Dermatology program, School of Anti-aging and regenerative Medicine, [2]Adviser, School of Anti-aging and regenerative Medicine, Mae Fah Luang University, 38/11-13 Asoke Place Building, Sukhumvit 21, Klong Toey Nua, Wattana, Bangkok, 10110, Thailand
**Corresponding author. E-mail: oasis_cloud@hotmail.com*

ABSTRACT

Riceberry is one of Thai agricultural products that contain high level of antioxidants. Previous studies showed that Riceberry extract was effective for inhibiting tyrosinase enzyme (Key enzyme of melanogenesis) in vitro. Hydroquinone is gold standard for treatment hypermelanosis. The mechanism of inhibit product melanin is inhibit Tyrosinase enzyme. Previous study showed that using of riceberry extract cream effectively reduce melanin production in human than cream base. Therefore, the objective of this research is to conduct a comparative of treatment of melasma with riceberry extract cream and 2% hydroquinone cream.The study was double-blind, randomized, controlled clinical Trial.24 Females with melasma on their face completed the study.Volunteers were randomly applied Riceberry extract cream and 2% Hydroquinone cream to one side of face every day (two times per day). This study involved 12 weeks of treatment. The skin color was measured before treatment and at 4th, 8th and 12th week by Mexameter MX18. Photographic images of each side of face were captured using visia camera before treatment and 12th week. The result revealed that riceberry extract cream and 2% Hydroquinone cream was found effective on melanin decreasing in the 4th week and level of mean melanin index continued to decrease at week 12th with the compare by conducting statistics t-test with significant number at 0.05. The result shows that the 2 treatments are no significant difference statistically. The dermatologists' evaluation and volunteer satisfaction rated mostly at high satisfied for both Riceberry extract cream and 2% Hydroquinone cream with no shown of significant difference statistically between both sides. No treatment-related side effects were observed. The conclusion is riceberry extract cream and 2% Hydroquinone cream have no difference on efficiency in melanin reduction for the treatment of melasma. And it also revealed that the riceberry extract cream has less side effect than 2% hydroquinone cream. Therefore it can be one of the alternative treatment for people with Melasma because they can use it for long term with less side effect.

Keywords: Riceberry extract cream/2% Hydroquinone cream/Melasma

Introduction

Melasma is the malfunction of the production of melanin which collects itself under the epidermis and/or dermis. It presents as macules and flat light to dark brown or grayish patches with irregular borders. Melasma doesn't come with birth but it collects and spreads itself. It appears on the areas that expose to the sun which are forehead, cheeks, chin and upper lip and it is more common in females than in males especially in the middle-aged (between the age of 30 and 40). Reason of casuing Melasma isn't clear but maybe with many factors together including UVA UVB from the sun which is believed to be the main reason that stimulates the melasma. Hormones especially Estrogen and Progesterone, emotion,cosmetics, pregnancy and inherit are also the reasons. The skin with Melasma has the same amount of melanocytes but with more intense working causes the increase in melanin.

Rice berry is a popular breed of rice with a very high anti-oxidant which can control Tyrosinase Enzyme which is the key to control the production of Melanin.Hydroquinone is the aromatic organic compound which can be found in nature from bombardier beetles which is a type of beetle and tagaricus hondensis which is a type of mushroom. The Hydroquinone cream will reduce or stop the production of melanin by melanocyte which reduce in melanin andcuretheMelasma.

The published research about rice berry compared to cream base and the suppression of Tyrosinase enzyme showed that the rice berry extract cream can effectively reduce the mean melanin index and also can develop into lightening skin product. Hydroquinone is a very popular intreating melasma. This research has been conducted to compare between the treatment of melasma with riceberry extract cream and 2% hydroquinone cream.

Objective

The objective of this study is to conduct a comparative of treatment of melasma with riceberry extract cream and 2% hydroquinone cream.

Material and Methods

The samples consist of females who has epidermal,deep and mixed melasma type with in age range of 20-60 years old with Fitzpatrick skin type 3-5.Exclusion criteria are whom used to apply Ionto treatment , hormones treatment or whitening drug, during pregnancy or breastfeed, has uncontrolled disease , has chemotherapy , smoker , highly expose to the sun ray and including other conditions that can leave the dark spot. This research is prospective clinical trial. The process will start from evaluation of melasma by the dermatologists and then take photo of before and after. Next measure the melanin with mexameter with mean melanin index and evaluate the MASI (melasma area and severity index) with the 2 dermatologists who are not involving with the result of the research. 24samples were given the sunblock and 2 facial creams to apply on the left and right side of the face. The dermatologists evaluate the side effect every 4th 8th 12th week and evaluate the satisfactory in the 12th week.

Data analysis

Data were analyzed using Pair t-test and satisfactory evaluation of treatment with Fisher‘s exact test and set the reliance at 95% (P value=0.05).

Result

30 healthy subjects were enrolled in this study. 24 volunteers completed the study. 6 volunteers left the study due to non-adhesion to the protocol. Table 1 shows comparison of mean melanin index of face that applied 2% Hydroquinone cream before and after treatment. Mean melanin index continued to decrease with statistically significant from 4th, 8th and 12th week with the p-value of 0.05. Table 2 shows comparison of mean melanin index of face that applied Riceberry extract cream before and after treatment. Mean melanin index continued to decrease from 4th to 12th week and continued to decrease with statistically significant at 12th with the p-value of 0.05. Figure 1 Linear graph shows the comparison of paired differences of mean melanin index of face that applied rice berry extract cream with 2% Hydroquinone cream which the level decreased with no significant difference statistically. The dermatologists’ evaluation and volunteer satisfaction rated mostly at high satisfied for both Riceberry extract cream and 2% Hydroquinone cream with no shown of significant difference between both sides (Figure 3 and Figure 4).

	Mean±SD	**Paired Differences**	**p-value**
Before treatment	248.13±59.02	3.75±3.62	<0.001*
After 4th week	244.38±58.46		
Before treatment	248.13±59.02	10.56±24.83	0.048*
After 8th week	237.57±63.94		
Before treatment	248.13±59.02	18.18±16.53	<0.001*
After 12th week	229.95±55.71		

Table 1: Comparison of mean melanin index of upper face that applied 2% Hydroquinone before and after treatment

	Mean±SD	**Paired Differences**	**p-value**
Before treatment	272.13±59.00	7.98±38.08	0.315
After 4th week	264.14±52.42		
Before treatment	272.13±59.00	13.04±32.09	0.058
After 8th week	259.09±51.11		
Before treatment	272.13±59.00	20.61±32.67	0.005*
After12th week	251.52±49.56		

Table 2 Comparison of maen melanin index of upper face that applied Riceberry extract cream before and after treatment

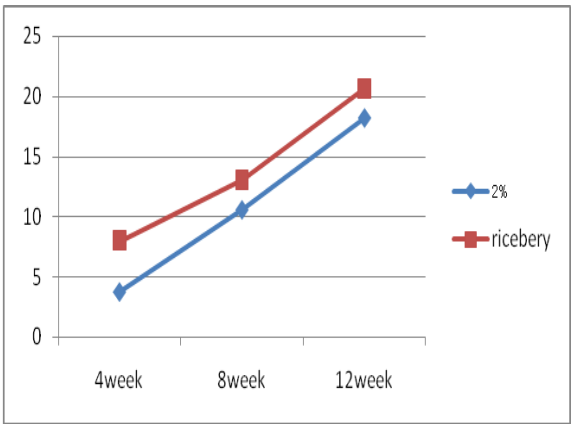


Figure 1: Linear graph shows comparison of paired difference of mean melanin index of the face that applied 2%Hydroquinone cream with Riceberry extract cream (p-value <0.05)

Table 3 shows comparison of MASI score that applied 2% Hydroquinone cream before and after treatment. MASI score continued to decrease with statistically significant from 4th, 8th and 12th week with the p-value of 0.05. Table 4 shows comparison of MASI score that applied Riceberry extract cream before and after treatment continued to decrease with statistically significant from 4t , 8th and 12th week with the p-value of 0.05. Figure 2 Linear graph shows the comparison of paired differences of MASI score that applied rice berry extract cream with 2% Hydroquinone cream which the level decreased with no significant difference statistically.

	Mean±SD	**Paired Differences**	**p-value**
Before treatment	5.55±1.85	1.00±1.25	0.08*
After 4th week	4.55±1.97		
Before treatment	5.55±1.85	2.19±1.38	<0.001*
After 8th week	3.36±1.81		
Before treatment	5.55±1.85	2.89±1.41	<0.001*
After 12th week	2.66±0.95		

Table 3: Comparison of MASI score that applied 2% Hydroquinone before and after treatment

	Mean±SD	**Paired Differences**	**p-value**
Before treatment	5.42±1.85	1.51±1.16	<0.001*
After 4th week	3.91±1.69		
Before treatment	5.42±1.85	2.44±1.31	<0.001*
After 8th week	2.98±1.33		
Before treatment	5.42±1.85	2.89±1.73	<0.001*
After 12th week	2.53±1.15		

Table 4 Comparison of MASI score that applied Riceberry extract cream before and after treatment

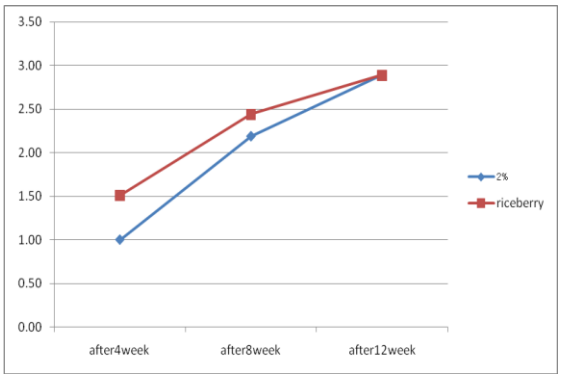


Figure 2: Linear graph shows comparison of paired difference of MASI score that applied 2%Hydroquinone cream with Riceberry extract cream (p-value <0.05)

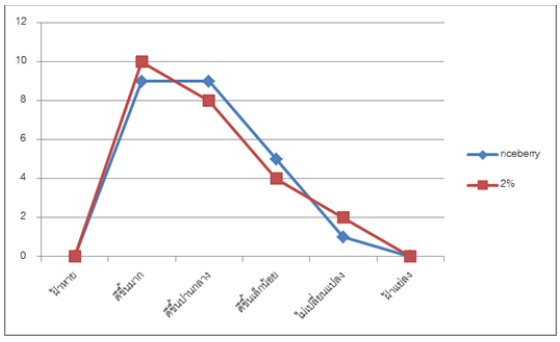


Figure 3: Linear graphs show dermatologist evaluation score of Riceberry extract cream compare with 2%Hydroquinone cream

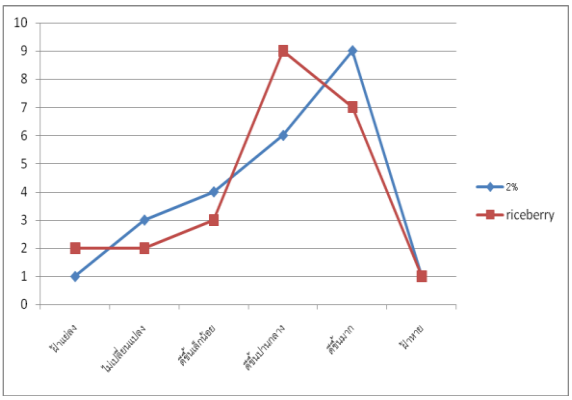
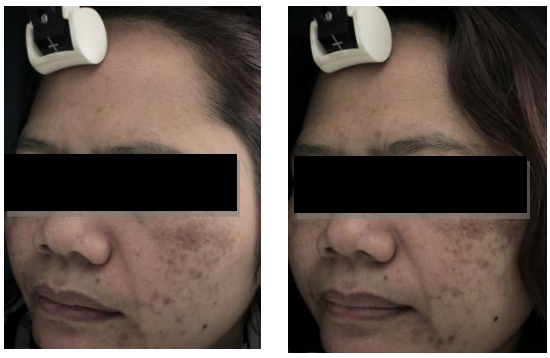


Figure 4: Linear graphs show volunteers evaluation score of Riceberry extract cream compare with 2%Hydroquinone cream

Riceberry extract cream

Before After 12 week treatment



2% Hydroquinone cream

Before After 12 week treatment

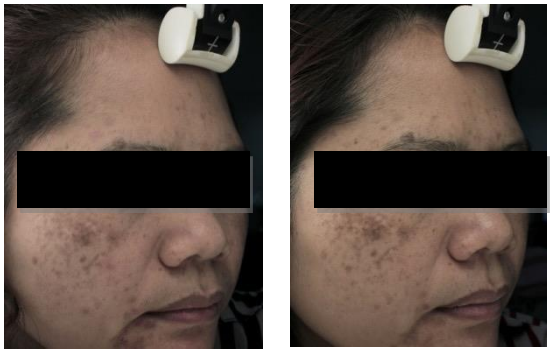


Figure 5: To compare photographs of Visia Camera between treatment by Riceberry extract cream and2% Hydroquinone cream

Discussion

In this study we evaluated the efficacy of Riceberry extract cream compare with 2% Hydroquinone cream for Melasma. When analyzing the reduction of mean melanin index of cheek before and after treatment, there was a decrease in mean melanin index compare to baseline in both sides. When compare between both sides: Riceberry extract cream and 2% Hydroquinone cream was found effective on melanin decreasing in the 4th week and level of mean melanin index continued to decrease at week 12th. And Riceberry extract cream and 2%Hydroquinone produced a noticeable reduction in melanin with statistically significant after 12th week. This finding was related with previous researches that demonstrated the inhibition of melanin formation by Riceberry extract in B16 melanoma 4A5 cells (Miyazawa, *et al*., 2013) , effect on tyrosinase inhibition and antioxidant effect of Riceberry extract (Kitsada, *et al.*, 2013). And previous researches that demonstrated the inhibition of melanin formation mechanism of depigmentation by hydroquinone. The present study showed that hydroquinone preferentially affected the nonfollicular and follicular melanocyte system. It caused decreased formation of melanosomes, a marked alteration in the internal structure of melanosomes, an increased degradation of melanosomes , and finally a destruction of membranous organelles in the melanocytes.(Jimbow K et al,1974).

In contrast to the decrease of mean melanin index, the dermatologists' evaluation and volunteer satisfaction were rated mostly at high satisfied and found no significant difference statistically between both sides. These result same as previous study. (Mataeng,2014)

The study shows that the side effects which appeared on the samples are red, itchy, dry and exfoliative skin with no significant difference statistically. It can be assumed that the reasons are the weather and sunlight of Thailand. Moreover, some samples who went abroad where the weather is dry which leads them to get dry and exfoliative skin and this side effect appeared equally on the whole face and with both cheeks which are applied riceberry extract cream and 2% hydroquinone cream. Accordingly it can be assumed that these side effects are not related to both cream but because of the confounding factors that have presented before. After giving advices such as avoid washing their face with warm water and following up the sample, the result is that the dry and exfoliative skin disappeared after a week and there is no more side effect.

Summary

The results of the study are that there is no significant difference statistically with the fading of Melasma between the treatment of riceberry extract cream and 2% hydroquinone cream by examine the information from the mexameter with mean melanin index, the MASI (melasma area and severity index) and the satisfactory of the dermatologists. The results also show that the riceberry extract cream has less side effect than 2% hydroquinone cream so it can be one of the alternative treatment for people with Melasma because they can use it for long term with less side effect.

Suggestion

For further studies giving longer periods to see wheather it can reduce the intensity and damage of Melasma or not, following the samples to see the effect after applying cream or the coming back of Melasma, the specific time of treatment, the intensity of rice berry which gives the best effect, less side effect and the results after pausing treatment from rice berry extract cream should be include.

References

Te-Sheng, C. An Updated Review of Tyrosinase Inhibitors. *International Journal of Molecular Sciences*.2009;10(6):2440-75.

Gillbro, J.M., Olsson, M.J. The melanogenesis and Mechanisms of skin-lightening agents existing and new approaches. *International Journal of Cosmetic Science*.2011; 33(3):210-21.

Leardkamolkarna, V, Thongthepb, W, Suttiarpornc, P,Kongkachuichaib, R, Wongpornchai, S, Wanavijitr, A. Chemopreventive properties of the bran extracted from a newly-developed Thai rice: The Riceberry. *Food chemistry*.2011;125(3):978–985.

Pitija, K, Nakornriab, M, Sriseadka, T, Vanavichit, A,Wongpornchai, S. Anthocyanin content &antioxidant capacity in bran extracts of some Thai black rice varieties.*Internationa Journal of Food Science &Technology*. 2013;48(2):300-308.

Miyazawa M, Oshima T, Koshio K, Itsuzaki Y, Anzai J. Tyrosinase inhibitor from black rice bran. *Journal Of Agricultural And Food Chemistry*.2013;51(24):6953-6.

Kitsada P, Nakornriab M, Tinakorn S, Apichart V, Suganya W, International Journal of Food Science Technology. 02/2013; 48(2):300-308.

Jimbow K, Obata H, Pathak M, Journal of Investigative Dermatology 05/1974; 62(4):436-4.

An Efficacy Assessment of Shallot Extract Gel for Adjunctive Therapy of Acne Vulgaris

Thanyaporn Hengpongthorn[1*] and Tanomkij Pawcsuntorn[2]

[1]Student in Master of science in Dermatology program, School of Anti-aging and regenerative Medicine, Mae Fah Luang University, 38/11-13 Asoke Place Building, Sukhumvit 21, Klong Toey Nua, Wattana, Bangkok, 10110, Thailand
[2]Adviser, School of Anti-aging and regenerative Medicine, Mae Fah Luang University, 38/11-13 Asoke Place Building, Sukhumvit 21, Klong Toey Nua, Wattana, Bangkok, 10110, Thailand
**Corresponding author. E-mail: giftzayaya@gmail.com*

Abstract

Acne vulgaris is a disorder of pilosebaceous unit. It is one of the most common skin disorders that mainly affects adolescent. It is necessary to find the cause and identify triggers in individual patients. Current treatment has modalities to treat the pathogenesis of acne but there are some disadvantages such as antibiotics associated drug-resistant bacteria, adverse effect of retinoids and drug allergy. Several studies show the properties of shallot extract or *Allium ascalonicum L.* are antioxidants, antibacterial and anti-inflammation but there was no study of shallot extract gel for acne vulgaris treatment. Therefore, this study aimed to determine the clinical effectiveness and safety assessment of topical shallot extract gel for adjunctive therapy of acne vulgaris. Thirty participants, men and women who aged between 18-50 years old, with mild to moderate grading of acne vulgaris were enrolled and assigned topical shallot extract gel apply on whole faces. Participants were instructed to apply the gels twice a day for 8 weeks and were evaluated the efficacy by lesions counting for each type of acnes, acne grading scale, side effects and subject satisfaction.25 subjects completed the study. The average age was 26.32 ± 5.281 years old. Almost of subjects were female (64%). The result revealed the lesion counting of non-inflammatory acnes significantly decrease from baseline at the fourth week and the lesion counting of inflammatory acnes was significantly decrease from baseline at the fourth week of the study. The severity of acne significantly improved at the fourth week. The adverse effect, only mild peeling skin was observed and improved with hydration. Almost of the participants were satisfied with the result of topical shallot extract gel at the eighth week of the study (96%). As results, topical shallot extract gel is effective for treat acne vulgaris both non-inflammatory and inflammatory acnes and improve the severity of acne with minimal adverse effects.

Keywords : Acne vulgaris/ Adjunctive therapy/ Shallot/ Shallot extract/ *Allium ascalonicum L./* Gel

Introduction

Acne vulgaris is a chronic disorder of pilosebaceous unit caused by follicular epidermal hyperproliferation, Excess sebum production, *Propionibacterium acnes* bacteria, inflammation and Hormonal effects. It can effect patients from childhood to adulthood, most often occurring in adolescents. The therapeutics modalities have been designed including topical agents and systemic therapy but current treatment has some disadvantages such as antibiotics associated drug-resistant bacteria, adverse effect of retinoids and drug allergy. There are several studies showed the properties of shallot extract or *Allium ascalonicum L.* which are antioxidants, antibacterial and anti-inflammation. Many patients used this alternative treatment of acne vulgaris and found that there was effective treatment without seriously adverse effect but there was no study of shallot extract gel for acne vulgaris treatment

Objective

This study aimed to determine the clinical effectiveness and safety assessment of topical shallot extract gel for adjunctive therapy of acne vulgaris.

Materials and methods

Research methodology

Thirty participants, men and women who aged between 18-50 years old with mild to moderate grading of acne vulgaris (acne grading scale by Dermatological Society of Thailand) were enrolled and assigned topical shallot extract gel apply on whole faces. Participants were instructed to apply the gel twice a day for 8 weeks and follow-up every 2 weeks. The study evaluated the efficacy by lesions counting for non-inflammatory and

inflammatory acnes, acne grading scale, side effects and subject satisfaction at 8th week by 2 independent doctors.

Statistical Analysis

Volunteers' research profile data was expressed by descriptive statistical analysis to provide descriptive information. Mean and standard deviation was used for continuous variables and as percentages for discrete variables.

Comparison of numbers of lesion counting of each types of acnes in topical shallot extract gel used repeated measure ANOVA between baseline and 2nd, 4th, 6th and 8th week. Data was analysed by SPSS. P-value less than 0.05 was considered statistically significance.

Comparison of acne grading scale between baseline and week 2nd, 4th, 6th and 8th week used signed ranks test to compare the mean of acne grading scale. Data was analysed by SPSS. P-value less than 0.05 was considered statistically significance.

Descriptive statistics were used for expressed the side effects and subject satisfaction at 8th week.

Results

From 30 participants, 25 subjects completed the study. The average age was 26.32 ± 5.281 years old. Almost of subjects were female (64%). The average age of onset of participants was 19.04 ± 5.358 years old and the average of duration of acne was 7.28 ± 3.921 years.

For lesion counting, the study revealed that the non-inflammatory type of acne vulgaris was significantly decrease the numbers of the lesions at 4th, 6th and 8th week when compare with baseline. (p-value < 0.05 respectively) The data are summarized in table 1 and figure 1.

Table 1 The comparison of numbers of lesion counting of non-inflammatory acnes.

Time	Mean ± SD	Mean difference	P-value
baseline - 2nd week	28.66 ± 14.55 27.50 ± 14.41	1.160	0.159
baseline - 4th week	28.66 ± 14.55 26.50 ± 14.13	2.160	0.016
baseline - 6th week	28.66 ± 14.55 24.86 ± 14.13	3.800	<0.001
baseline - 8th week	28.66 ± 14.55 24.12 ± 14.13	0.760	<0.001

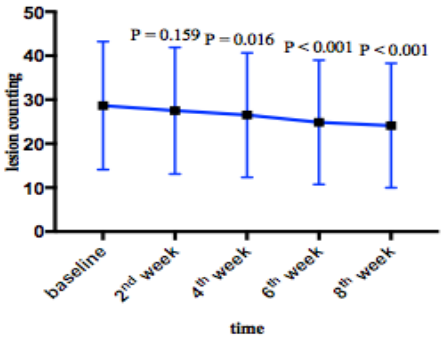


Figure 1 Linear graph compared difference mean change of lesion counting of non-inflammatory acne and P-value of each week compare with baseline

In inflammatory type of acne, the result revealed that the lesions counting was significantly decrease at 4th, 6th and 8th week when compare with baseline (p-value < 0.05 respectively) The data are summarized in table 2 and figure 2.

Table 2 The comparison of numbers of lesion counting of inflammatory type of acne vulgaris.

Time	Mean ± SD	Mean difference	P-value
baseline - 2nd week	9.86 ± 5.33 9.46 ± 5.32	0.400	0.691
baseline - 4th week	9.86 ± 5.33 8.58 ± 5.45	1.280	0.003
baseline - 6th week	9.86 ± 5.33 7.38 ± 5.19	2.480	<0.001
baseline - 8th week	9.86 ± 5.33 6.46 ± 4.99	3.400	<0.001

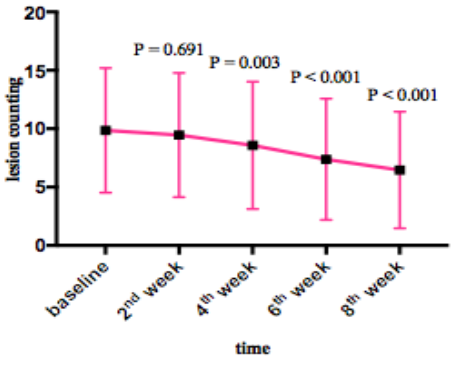


Figure 2 Linear graph compared difference mean change of lesion counting of inflammatory acne and P-value of each week compare with baseline

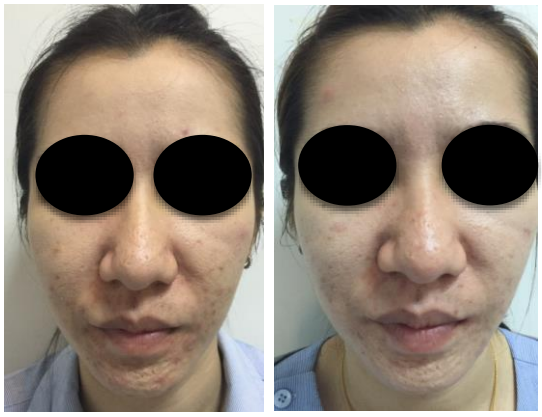


Figure 3 The picture of some volunteer compare between baseline (left) and 8th week (right).

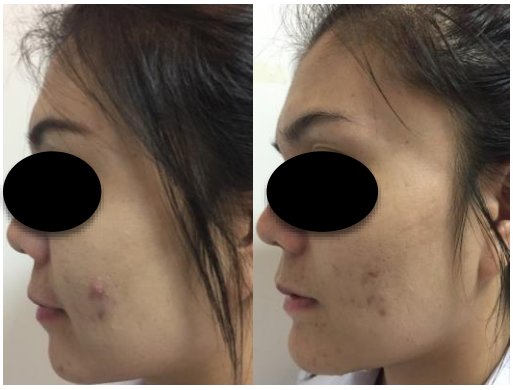


Figure 4 The picture of some volunteer compare between baseline (left) and 8th week (right).

The outcome of acne grading scale showed that the severity of acne vulgaris was significantly improved at 4th, 6th and 8th week. (p-value < 0.05 respectively) The data are summarized in table 3.

Table 3 the comparison of acne grading scale between baseline and 2nd, 4th, 6th and 8th week.

time	mean ± SD	P-value (compare with baseline)
baseline	1.48 ± 0.51	-
2nd week	1.42 ± 0.47	0.408
4th week	1.30 ± 0.46	0.034
6th week	1.22 ± 0.41	0.011
8th week	1.20 ± 0.41	0.008

The all adverse effects were observed and found 1 person had temporarily peeling skin at 2nd week of treatment. This effect was improved by hydration.

After the participants completed the study. Satisfaction was evaluated and found almost of subjects satisfied the treatment of acne vulgaris by shallot extract gel (96%). 48 percent of them satisfied in high level and 24 percent of them satisfied in each medium and low levels. 4 percent of subjects were indifferent between before and after treatment.

Discussion

This study aimed to treat the acne vulgaris by stop the pathogenesis of the disease. Several studies showed the properties of shallot extract or *Allium ascalonicum L.* are antioxidant, anti-inflammatory and antibacterial that had tendency to treat the pathogenesis of acne vulgaris. Some people used this herb for treat the acne vulgaris and found the effectiveness without seriously complication but there was no study of this plant for acne treatment.

From the study of Fattorusso et. al. in 2002 found the property of antioxidant in *Allium ascalonicum L.* There are rich of flavonoids and saponins that had capability to remove free radicals and anti-inflammation. The study also found the property of antibacterial function by inhibit the growth of some bacterias especially *Staphylococcus aureus.* Moreover Nishino et. al. in 2015 revealed flavonoids can inhibit *Staphylococcus epidermis* suggested their efficacy in treating staphylococcal infection. Mohammadi-Motlagh et. al. in 2010 studied the anti-inflammatory effect of *Allium ascalonicum L.* found the ability of decrease the inflammation by inhibit the leukocytes and inhibit the function of anti-inflammatory enzymes. These results suggesting that shallot extract or *Allium ascalonicum L.* may be effectiveness in treatment of acne vulgaris.

From this study found that shallot extract gel has a potent and topically effective agent to treat the acne vulgaris. In the treatment of non-inflammatory acnes found the statistically significant efficacy in treatment since the fourth week and the inflammatory acnes since the fourth week too. The result suggested that shallot extract gel or *Allium ascalonicum L.* may have ability in follicular epidermal hyperkeratinization.

In severity of acne, this study revealed the statistically significant improvement of severity of acne since the fourth week. This result may suggested the efficacy of shallot extract gel for treatment acne vulgaris.

There is only mild peeling skin as a minor adverse effect and improvement by hydration. No seriously or other adverse effects was observed. The study suggested that the shallot extract gel have a safety assessment for preliminary application in first two months.

Almost of the participants were satisfied with the result of topical shallot extract gel at the eighth week of the study. This satisfaction is the important factor of choosing the alternative treatment instead of the conventional treatment.

Conclusion

Topical shallot extract gel is effective and safe in treatment of both non-inflammatory and inflammatory acne vulgaris. Only minor adverse effect was observed.

Suggestion

Furthermore, The study should compare the shallot extract gel with the standard medication and increase the duration of treatment for evaluate the efficacy, adverse effects and progression of the disease.

References

Fattorusso, E., Iorizzi,M., Lanzotti,V. & Taglialatela-scafati, O. (2002). Chemical composition of shallot (Allium ascalonicum Hort.). Journal of Agricultural and Food Chemistry, 50(20), 5686-5690.

Joshi,U., Gadge, A.S.,D'Mello, .,Sinha,R.,Srivastava,S. & Govil, G.(2011). Anti-inflammatry, antioxidant and anticancer activity of Quercetin and its analogues. International Journal of Research in Pharmaceutical and Biomedical Sciences, 2(4), 1756-1766.

Leelarungrayub, N.,Rattanapanone, V., Chanarat, N.& Gebicki, J.m.(2006). Quantitative evaluation of the antioxidant properties of garlic and shallot preparations.Basic nutritional investigation, 22, 266–274.

Mnayer, D., Fabiano-Tixier, A., Petitcolas, E., Hamieh, T., Nancy, N., Ferrant, C., Fernandez, X. &Chemat, F.(2014). Chemical Composition, Antibacterial and Antioxidant Activities of Six Essentials Oils from the Alliaceae Family.Molecules 2014, 19, 20034-20053.

Mohammadi-Motlagh, H., Mostafaie, A. & Mansouri, . (2010). Anticancer and anti-inflammatory activities of shallot (Allium ascalonicum) extract. Archives of Medical Science, 7(1), 38-44.

Nishino, C., Enoki, N., Tawata, S., Mori, A., Kobayashi, K. & Fukushima, M. (2015). Antibacterial Activity of Flavonoids against Staphylococcus epidermidis, a Skin Bacterium. Agricultural and Biological Chemistry, 51(1), 139-143.

Zaenglein, A.L., Graber. E.M., Thiboutot, D.M. & Strauss, J.S.(2008). Acne Vulgaris and Acneiform Eruption. In Fitzpatrick's Dermatology in general medicine (1st, pp.690-703). USA: McGraw- Hill.

Zaenglein, A.L. & Thiboutot, D.M. (2008). Acne Vulgaris. In: Bolognia JL, Jorizzo JL, Rapini RP, eds. Dermatology 2 nd ed. Philadelphia: Mosby Elsevier

Anti-cancer Effects of Artabotrys Siamensis on Human Leukemia K562 and RAJI Cell Lines

Kitti Pumiputavon[1], Tanawat Chaowaskul[1], Chalermpong Saenjum[2], Maslin Osathanunkul[1], Boonsong Wungsintaweekul[3], Kriangkrai Chawansuntati[4], Jiraprapa Wipasa[4*] and Pathrapol Lithanatudom[1*]

Institutional mailing address:
[1] *Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, 50200, Thailand*
[2] *Department of Pharmaceutical Science, Faculty of Pharmacy, Chiang Mai University, 50200, Thailand*
[3] *School of Pharmacy, Walailak University, Nakhon Si Thammarat, 80160, Thailand*
[4] *Research Institutes for Health Sciences, Chiang Mai University, Chiang Mai, 50200, Thailand*
** Corresponding author*
Email: jwipasa@hotmail.com and pathrapol_li@hotmail.com

Abstract

Artabotrys siamensis is a species in the *Annonaceae* family which is of interest to be screened for anti-cancer activity in some cancer cell lines. Leukemia K562 and RAJI cell lines were incubated in the presence of crude extracted from the leaves of *Artabotrys siamensis* by methanol. Cells were analysed by Annexin V/propidium iodide double staining. Only the crude extract of *Artabotrys siamensis* leaves induced apoptosis in K562 and RAJI cell lines. The percentages of apoptotic cells were dependent on crude extract concentrations.

Introduction

Cancer is a serious illness that causes more than one million deaths around the world. It can be characterised by uncontrollable cell growth leading to cell spreading all over the body and vital organs (Anand *et al*., 2008). It can lead to morbidity and mortality eventually (Beeran *et al*., 2015). Previous studies have shown that the important factors of cancer development were external or environmental factors (90%), including tobacco (30%), unhealthy diet (35%), infectious organism (20%), radiation (10%) and stress and environmental pollutant (5%) (American Cancer Society USA, 2015). Moreover, others factors such as mutation and inherited genetic can be found in 10% of cancer patients (Anand et al., 2008). Cancer is the secondary cause of human death worldwide, higher than the numbers of AIDS, tuberculosis and malaria in 2012. Until now, more than 100 types of cancer have been discovered and have a tendency to increase (American Cancer Society, 2015). Effective cancer treatment is essentially required for patient care in the future. Common methods of cancer treatment, including surgery, chemotherapy and radiotherapy, have some limitations and critical side effects, resulting in cancer resistance and relapses (Gottesman *et al*., 2002). Hence, new strategies are required for the management and therapy of cancer.

Apoptosis or the process of programmed cell death is a stringent pathway for controlling and eliminating unwanted, old and injured cells from tissue. It is a great strategy to get rid of cancerous cell (Wong 2011). Apoptosis leads to morphological change, heterochromatin condensation, cell shrinkage and budding, loss of organelles in cytoplasm and formation of apoptotic bodies (Kerr 1972). Therefore, in the field of medical oncology, some natural compounds have the ability to induce apoptotic pathway in cancer cells. Natural products have been an excellent source of pharmaceutical products in human health care for thousands year. Crude extracts from plants are essential source for screening of apoptosis inducers. It has potential to be applied for cancer therapy.

The *Annonaceae* family is a tropical plant family of trees, shrubs and lianas. This species plays an important ecological role to species diversity in tropical zone around the world (Couvreur 2011). Interestingly, excellent active compounds can be isolated from all parts of the *Annonaceae* family (Aminimoghadamfarouj 2011). Thus, it is of interest to examine some species of rare genera in the *Annonaceae* family for bioactive compound with anti-cancer activity. In this study, the ability of crude extracted from *Artabotrys siamensis*, a species in the *Annonaceae* family, was evaluated for its ability to kill human myeloid leukemia cell lines (K562 and RAJI).

Materials and Methods

1. Materials

Roswell Park Memorial Institute medium (RPMI), fetal bovine serum (FBS), Penicillin-Streptomycin, L-Glutamine, Fungizone and 0.25% Trypsin-EDTA were purchased from Gibco-BRL, USA. Annexin V-FITC (Fluorescein Isothiocyanate) was purchased from ImmunoTools GmbH, Germany and biolegend inc, canada. HEPES was purchased from Merck Millipore, Germany. Sodium bicarbonate were purchased from

RCI LABSCAN, Thailand. Dimethyl sulfoxide (DMSO) and Ribonuclease A (RNase A) was purchased from Worthington Biological Corporation USA.

2. Cell lines and culture

Human cancer cell lines (K562 and RAJI) were maintained in RPMI containing 10 mM of HEPES, 1 mM of sodium bicarbonate, 10% fetal bovine serum (FBS), 100 IU/ml penicillin and 100 □g/ml streptomycin (RPMI complete media) at 37ºC in a humidified 5% CO_2 atmosphere.

3. Plant extraction

The dried powder of leaves of *Artabotrys siamensis* were incubated in methanol for 24h. Then solvent was collected and evaporated under vacuum in a rotary evaporator. All methanolic extract were dissolved in dimethyl sulfoxide (DMSO) at a concentration of 100 mg/ml. Finally, these extracts were diluted to 1000 µg/ml, 500 µg/ml, 250 µg/ml and 125 µg/ml in RPMI complete media.

4. Annexin V assay

Human cancer cell lines K562 and RAJI cell lines at 1 x 10^5 cells/ml were cultured in 24-well plates in the presence of various concentrations of methanolic extracts (1000 µg/ml, 500 µg/ml, 250 µg/ml and 125 µg/ml) for 24h. The quantification of apoptotic cells was measured by Annexin V-FITC (Fluorescein Isothiocyanate)/ PI (Propidium Iodide) double staining assay. At the end of incubation, cells were harvested and centrifuged at 1800 rpm for 8 min. The pellet was resuspended in 50 µl binding buffer containing 0.5 µl Annexin V-FITC and incubated at 4ºC for 30 min in the dark. PI (50 µg/ml) in 200 µl annexin binding buffer were added to each tube and incubated for 5 min. Finally, the cells were analysed by flow cytometry (CyAn ADP Analyzer, Beckman Coulter USA).

Results and discussion

The anticancer activity of crude extract on cancer cell lines was correlated with apoptosis. In normal cells, phosphatidylserine (PS) is ordinarily located at the inner side of plasma membrane. PS will translocate from the inner to the outer membrane when cells are induced to undergo apoptosis. Annexin V is a protein that binds specifically with high affinity to PS. From this ability, annexin V can be used to investigate the externalisation of PS on the outer membrane of apoptotic cells. Annexin V-FITC/PI double staining can be applied to confirm the apoptosis of cancer cell lines. Annexin V+/PI- (lower right quadrant) and Annexin V+/PI+ (upper right quadrant) cells determined early and late apoptotic cell populations, respectively. Other quadrants, annexin V-/PI- (lower left quadrant) and annexin V+/PI+ (lower left quadrant) determined live and necrotic cell populations, respectively. In DMSO control (DMSO-C), cancer cell lines did not show apoptosis induction, but treatment with crude extract (leaf of Artabotrys siamensis; LAS) showed variation of the percentages of apoptotic cells in a dose-dependent manner (Table 1 and Figure 1).

Induction of apoptosis in cancer cells is a strategy for cancer therapy. This study demonstrated the apoptotic activity of LAS on human leukemia K562 and RAJI cell lines. A previous study has shown that extract from leaf of *Artabotrys siamensis* had low anti-cancer activity on colorectal carcinoma (COLO-205), cervical carcinoma (Hela) and hepatoma (HeSG2) (Uthaisang *et al*., 2005). On the contrary, this study showed high anti-cancer activity of crude extract on K562 and RAJI cells. Increases of cells in early and late apoptosis phase when treated with LAS compared to DMSO-C were observed. Anti-cancer activity of species in the *Annonaceae* family such as *Polyalthia longifolia* (Verma *et al.,* 2008) and *Annona muricata* (Moghadamtousi *et al.,* 2014) have been reported. Results from our study suggest that *Artabotrys siamensis* may have some active compounds capable of killing cancer cell lines such as K562 and RAJI cells. Determination of active compound of *Artabotrys siamensis* warrants examination in the future.

Table 1. Percentages of apoptotic cells when K 562 or RAJI cell lines were incubated in methanolic extract of *Artabotrys siamensis* leaves.

	% Apoptotic cells (Sum of early and late apoptotic cells)				
	1000 µg/ml	500 µg/ml	250 µg/ml	125 µg/ml	DMSO
K562	85.1±6.68	62.4±10.22	27.0±1.75	7.3±1.66	5.0±0.14
RAJI	98.7±0.15	93.4±0.68	41.2±0.49	24.5±6.16	20.1±0.98

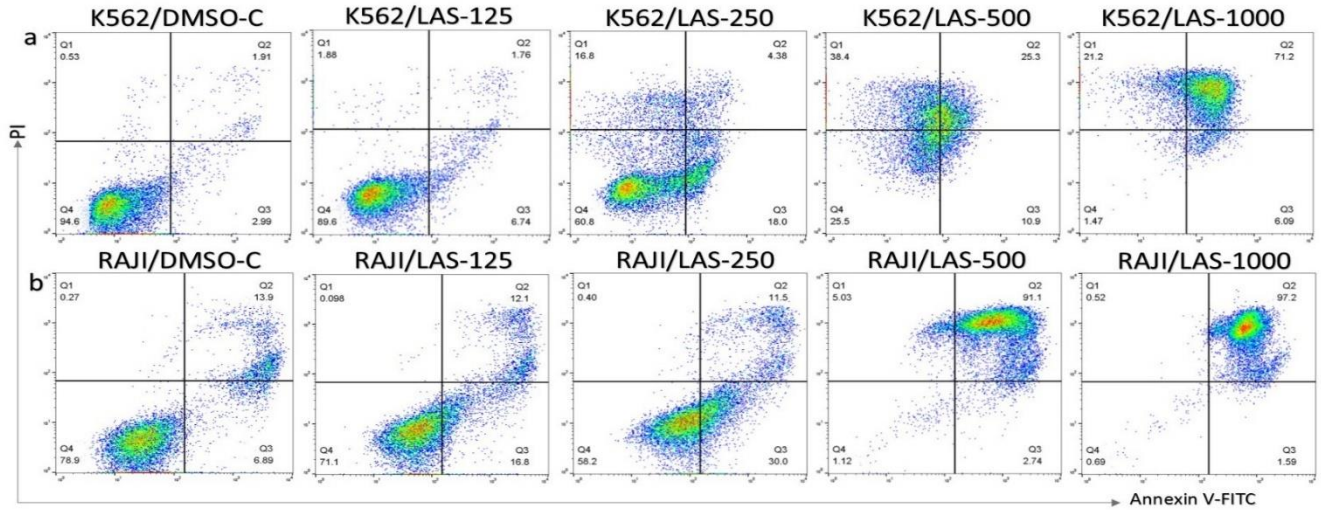


Figure. 1. Apoptosis induction of methanolic extract of *Artabotrys siamensis* leaves (LAS) in K562 (a) and RAJI (b) cell lines.

Conclusion

We found that extract from leaves of *Artabotrys siamensis* could induce human leukemia K562 and RAJI cells to undergo apoptosis. Further investigation of LAS as a source of pharmacologically active agent, and identification and purification of bioactive compounds of this plant is warranted.

Acknowledgments

We are thankful to the Department of Biology, Faculty of Science, Chiang Mai University and the Research Institutes for Health Sciences, Chiang Mai University for providing resources to conduct this study. Authors thank all research assistants at the Research Institutes for Health Sciences, Chiang Mai University for their help in all aspects of research.

References

American Cancer Society USA, (2015). Global Cancer Facts & Figures 3rd Edition. http://www.cancer.org/research/cancerfactsstatistics/cancerfactsfigures2015/ (accessed 07/04/16)

Anand, P., Kunnumakara, A.B., Sundaram, C., Harikumar, K.B., Tharakan, S.T., Lai, O.S., Sung, B.Y., Aggarwal, B.B., (2008). Cancer is a Preventable Disease that Requires Major Lifestyle Changes. Pharm Res-Dord 25(9), 2097-2116.

Beeran, A.A., Maliyakkal, N., Rao, C.M., Udupa, N., (2015). The enriched fraction of Elephantopus scaber Triggers apoptosis and inhibits multi-drug resistance transporters in human \ epithelial cancer cells. Pharmacogn Mag 11(42), 257-268.

Gottesman, M.M., Fojo, T., Bates, S.E., 2002. Multidrug resistance in cancer: role of ATP-dependent transporters. Nat Rev Cancer 2(1), 48-58.

Kerr, J.F., Wyllie, A.H., Currie, A.R., 1972. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br J Cancer 26(4), 239-257.

Wong, R.S., 2011. Apoptosis in cancer: from pathogenesis to treatment. J Exp Clin Cancer Res 30, 87.

Aminimoghadamfarouj, N., Nematollahi, A., Wiart, C., 2011. Annonaceae: bio-resource for tomorrow's drug discovery. J Asian Nat Prod Res 13(5), 465-476.

Couvreur, T.L.P., Pirie, M.D., Chatrou, L.W., Saunders, R.M.K., Su, Y.C.F., Richardson, J.E., Erkens, R.H.J., 2011. Early evolutionary history of the flowering plant family Annonaceae: steadydiversification and boreotropical geodispersal. J Biogeogr 38(4), 664-680.

Uthaisang, W. and Khawsak, P. (2005). Cytotoxicity of crude extracts from Allamanda Cathartica. Guaiacum officinale and Artabotrys siamensis to some cancer cells. Journal of Medicine and Health Sciences. Faculty of Medicine, 12 (2), 59-69.

Moghadamtousi, S. Z., Kadir, H.A., Paydar, M., Rouhollahi, M. and Karimian, H. (2014). Annona muricata leaves induced apoptosis in A549 cells through mitochondrial-mediated pathway and involvement of NF-κB. BMC Complementary and Alternative Medicine, 14, 299.

.

A Randomized Clinical Trial of 5% Topical Minoxidil versus 10% Dihydrooxyresveratrol from Artocarpus Lakoocha Roxbin the Treatment of Androgenetic Alopecia in Men

Patpapa Tungtanayongsuk[1] and Wipapen Chokdeesumrit[2*]

[1,2] *Mas ter of Science Program in Dermatology,*
School of Anti-aging and Regenerative Medicine,Mae Fah Luang university,Thailand
[*] *Corresponding author.E-mail: xiu_xiu_bo@hotmail.com*

Abstract

Dihydrooxyresveratrol, a new derivative of Oxyresveratrol, indicated more potent promote hair follicle keratinocyte proliferation compared to Oxyresveratrol. Previous studies showed 5% concentration of Dihydrooxyresveratrol can effectively promote hair growth equally to 5% topical minoxidil. In this study we compare the efficacy of 10 % Dihydrooxyresveratrol and 5% topical minoxidil for hair growth.The aim of this research is to compare efficacy of 10% Dihydrooxyresveratrol and 5% topical minoxidil for hair growth.There are Twenty male subjects (aged -1860 years old) with androgenetic alopecia were enrolled and randomly assigned 10%Dihydrooxyresveratrol on one side of head and 5%topical minoxidil on the other side. Participants were instructed to apply them twice a day for 12 weeks. Participants were followed up every 4 weeks. Evaluation was performed every 4-week interval using hair diameter and hair number measured by Folliscope, photographs from VISIA® and records of side effects.Volunteers' satisfaction was assessed by questionnaires at 12th week.The results found that eighteen subjects completed the study.Global photographic scores, hair diameter and number in 10%Dihydrooxyresveratrol group(66.94±9.77µm and 43.5±33.1 hairs0.36/cm^2) were not significantly different to 5%topical minoxidil group(62.61±7.75µm and 41±89.352.hairs0.36/cm^2) at week 12 with minor side effects. Both groups demonstrated statistically significant hair diameter from the baselines at 4th week, 8th week, and 12th week, respectively. However, global photographic scores between both sides revealed no statistically significant in results. Additionally,Satisfaction scores in 10%Dihydrooxyresveratrol group were not significantly different to 5%topical minoxidil group.Side effects were recognized only mild in both groups. Conclusion : This suggested 10%Dihydrooxyresveratrol is effective on hair growth.

***Keywords*:** Dihydrooxyresveratrol/Minoxidil/hair

Introduction

Hair and hair styles can help human attractive.The thickness and healthy of human hair are the desirable of individual. Nowadays alopecia is major concern problem in population.Treatment of alopecia is currently more interest. (Thaichinda, 2008**)** In the treatment of alopecia,Current drug approved by The Food and Drug Administration of the United state and Thailand for hair growth is Minoxidil.It is approved for the treatment of Androgenetic alopecia (Androgenetic alopecia) (Rogers & Avram, 2008).In term of side effect minoxidil is also common. (Guarneri & Cannavò, 2013.Nowadays many peoples use botanical product for alopecia.

The Government Pharmaceutical organization by Dr.Piyaporn and colleagues conducted the study on hair growth.They found that Dihydrooxyresveratrol can promote hair follicle keratinocytes proliferation. Dihydrooxyresveratrol, a new derivative of Oxyresveratrol, indicated more potent promote hair follicle keratinocyte proliferation compared to Oxyresveratrol. Previous studies showed 5% concentration of Dihydrooxyresveratrol can effectively promote hair growth equally to 5% topical minoxidil. In this study we compare the efficacy of 10 % Dihydrooxyresveratrol and 5% topical minoxidil for hair growth.The study is to determine the efficacy of 10%Dihydrooxyresveratrol from *ARTOCARPUS LAKOOCHA* ROXB. compared to 5%topical minoxidil for hair growth.Global photographic score,hair diameter and hair numbers are evaluated in this study.Satisfaction score and side effect are also evaluated.

Objective

To determine the efficacy of 0%Dihydrooxyresveratrol compared to 5%topical minoxidil for hair growth. Satisfaction score and side effect are also evaluated.

Materials and Methods

Twenty male subjects (age18 - 60 years old) with androgenetic alopecia in Norwood-Hamilton scale I-III were enrolled and randomly assigned 10%Dihydrooxyresveratrol on one side of head and 5%topical minoxidil on the other side. Participants were instructed to apply them twice a day for 1 2 weeks. Participants were followed up every 4 weeks.Hair diameter and hair numbers were assessed by Follioscope. Global photographic scores were assessed by VISIA®.Side effect were also evaluated by medical researchers and volunteers.After 12 weeks satisfaction score were assessed by volunteers.

Statistic for data analysis

Mean change of hair diameter and hair number in same side before and after applying drug were calculated by Pair T-test. Mean change of hair diameter and hair number to other side before and after applying drug were calculated by Unpaired t-test. Global photographic scores were calculated by Mann-Whitney U test. Satisfaction score and side effect were calculated by Fisher-Exact test.

Results

When compared the efficacy between 10%Dihydrooxyresveratrol and 5%topical minoxidil for hair growth found that Global photographic scores in 10% Dihydrooxyresveratrol group were not significantly different to 5%topical minoxidil group after 4th,8th and 12th weeks. ($p = 0.576$, $p = 0.842$ and $p = 0.668$) (Figure1).Eighteen volunteers completed the study.The average participant age was 40.33 ± 8.80 years old. Hair diameter and number in 10%Dihydrooxyresveratrol group(66.94+9.77µm and 43. 5 + 3 3 . 1 hairs0 . 3 6 / cm2) were not significantly different to 5%topical minoxidil group(62.61+7.75µ m and 41+89.352.hairs0.36/cm2) at 12th week .

Hair diameter in 10%Dihydrooxyresveratrol group were significantly superior to baseline after 4th weeks.($p < 0.001$) .In 5%topical minoxidil group that Hair diameter were significantly superior to baseline after 4th weeks. (From 37.33 ± 4.83 to 47.50 ± 3.33), ($p < 0.001$). Hair numbers in 10%Dihydrooxyresveratrol group and 5%topical minoxidil group were significantly superior to baseline after 4th weeks.($p = 0.014$ and $p= 0.024$). Compared differences of hair diameter showed 10% Dihydrooxyresveratrol were more effective than 5%topical minoxidil at 4th week, 8th week and 12th week but not statistic significant.($p = 0.844$, $p=0.100$,$p=0.556$), (Figure 2) .And paired differences of Hair numbers in 10%Dihydrooxyresveratrol group were not significantly different to 5%topical minoxidil group after 4th,8th and 12th weeks.(Figure3)

Additionally Satisfaction score in 10%Dihydrooxyresveratrol group were not significantly different to 5%topical minoxidil group after 12th weeks.) $p = 0.322$(.Side effect in 10%Dihydrooxyresveratrol group were not significantly different from 5%Minoxidil group ($p = 0.44$).Side effect were occurred temporarily mild pruritus and stinging.

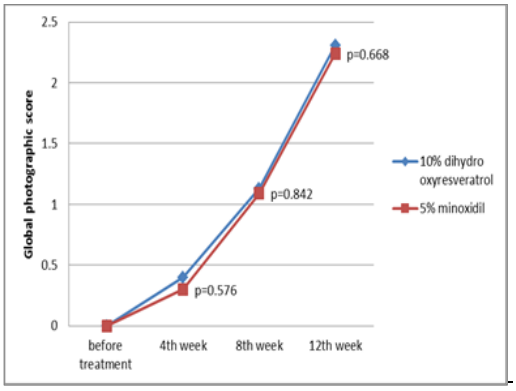
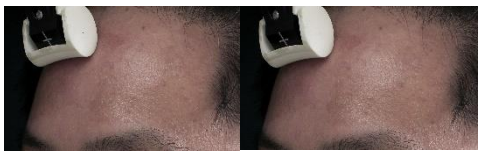


Figure1 Linear graphs showed comparison Global photographic scores between 10% Dihydrooxyresveratrol and 5%topical minoxidil.

Picture 1. Votunteer applied 10% Dihydrooxyresveratrol. Left is before applied at week .Right is after applied for 12 weeks



Picture 2. Votunteer applied 5% topical minoxidil . Left is before applied at week 1.Right is after applied for 12 weeks

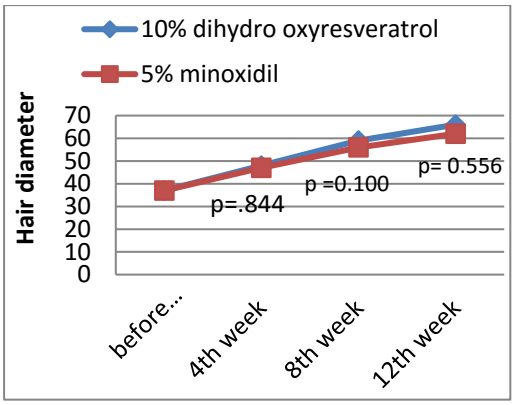
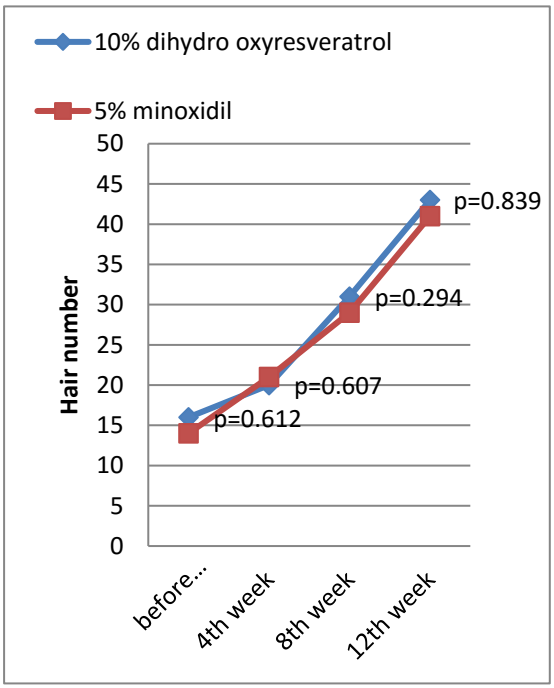


Figure 2 Linear graphs showed comparison Hair diameter between 10%Dihydrooxyresveratrol and 5%topical minoxidil.



Discussion

When analyzing hair growth from folliscope showed 10% Dihydrooxyresveratrol and 5% topical minoxidil were statistically significant in hair growth compared to Baseline since 4th weeks. Theses outcome were related to The Government Pharmaceutical organization by Dr.Piyaporn and colleagues.They were conducted study about hair growth in vitro.They found that Dihydrooxyresveratrol promote hair follicle keratinocyte proliferation.

The efficacy also increased to maximum observed at 12th week in both groups. After 12th week, the efficacy of 10%Dihydrooxyresveratrol from *ARTOCARPUS LAKOOCHA* ROXB. were not significantly different from 5%topical minoxidil for hair growth.

Primary outcome of this study found that Global photographic scores, hair diameter and number in 10%Dihydrooxyresveratrol group were not significantly different to 5%topical minoxidil group. The research outcomes that was evaluated visual evaluation. We used VISIA®, which compared photos in the same positioning and standardized lighting. Our finding, global photographic scores showed no significantly different between both groups.

In comparison to previous study of TEERASAK C. (TEERASAK , 2015) with different concentration of Dihydrooxyresveratrol, 5% in prior study showed equally effective. This possible explained by Dihydrooxyresveratrol also promote hair follicle keratinocyte proliferation in a dose-dependent manner.As a result that increasing the concentration of Dihydrooxyresveratrol to 10% were more effective because 10% Dihydrooxyresveratrol were statistically significant in hair growth compared to Baseline since 4th weeks.While 5%Dihydrooxyresveratrol significant in hair growth compared to Baseline since 8th weeks.

Moreover,Secondary outcome were Satisfaction score and side effect. Satisfaction score in 10%Dihydrooxyresveratrol group were not significantly different to 5%topical minoxidil group after 12th weeks. Side effect in 10%Dihydrooxyresveratrol group were not significantly different from 5%Minoxidil group.Side effect were occurred temporarily mild pruritus and stinging.Propylene glycol might be causative agent.

Summary

10%Dihydrooxyresveratrol is equally effective on hair growth to 5% topical minoxidil. And also safe with few local side effects.

Suggestion

The effectiveness on hair growth between 10%Dihydrooxyresveratrol and 5%Minoxidil in long term should be perform for more information about efficacy and side effect. Additionally,The advanced Study should be perform after drug withdrawal for more information about hair loss.

References

1. Rogers, E. N. & Avram, R. M. (2008). Medical treatments for male and female pattern hair loss. *J Am Acad Dermatol, 59*, 547-566.
2. Guarneri, C. & Cannavò, S. P. (2013). Erosive pustular dermatosis of scalp from topical

minoxidil 5% solution. *Int J Dermatol*, *52*(4), 507-509.

3. Abell, E. (1988). Histologic response to topically applied minoxidil in male- pattern alopecia. **Clin Dermatol, 6**, 191-194.
4. Baker, C. A., Uno, H. & Johnson, G. A. (1994). Minoxidil sulfation in the hair follicle.**Skin Pharmacol**, *7*, 335-339.
5. Campese, V. M. (1981). Minoxidil: A review of its pharmacological properties and therapeutic use. **Drugs**, *22*, 257-278.
6. Hordinsky, M. K., Wietgrefe, M. M., Sevenich, E., Hallgren, H. & Filipovich, A. M. (1985). Immune function in alopecia areata patients applying 3% topical minoxidil.**Clin Res, 33**, 646A.
7. Khoury, E. L., Price, V. H., Abdel-Salam, M. M., Stern, M. & Greenspan, J. S. (1992). Topical minoxidil in alopecia areata: No effect on the perifollicular lymphoid infiltration. **J Invest Dermatol, 99**, 40-47.
8. **Olsen**, E. A., Whiting, D. A., Savin, R., Rodgers, A., Johnson-Levonas, A. O., Round, E., Rotonda, J. & Kaufman, K. D. (2012). Global photographic assessment of men aged 18 to 60 years with male pattern hair loss receiving finasteride 1 mg or placebo.**J Am Acad Dermatol, 67**, 379-386.

A Randomized, Double-blind, Control Trial Study of the Efficacy of 5% Artocarpus Lakoocha Heartwood Extract Combined 4%Niacinamide Compare with 5% Artocarpus Lakoocha Heartwood Extract Alone in the Treatment of Axillary Hyperpigmentation in Thai Women

Kitchai Thungjakwaranun

Student in program Master of Science in Dermatology
School of Anti-Aging and Regenerative Medicine, Mae Fah Luang University, Thailand
Corresponding author. Email:kitchai_104@hotmail.com

Abstract

Axillary hyperpigmentation is a frequent cause of cosmetic consultations in women, there is no widely accepted treatment and its limitation of clinical trial. 5% Artocarpus lakoocha Heartwood Extract topical treatment and 4%Niacinamide topical treatment have been reported being effective in the treatment of Axillary hyperpigmentation but there is no clinical study that demonstrating the efficacy of 5%Artocarpus lakoocha Heartwood Extract Combined 4%Niacinamide in the Treatment of Axillary hyperpigmentation

Objective: The purpose of this study was to assess the efficacy of 5%Artocarpus lakoocha Heartwood Extract Combined 4%Niacinamide Compare with 5%Artocarpus lakoocha Heartwood Extract alone in the Treatment of Axillary hyperpigmentation in Thai women.

Materials and Methods: twenty-one participants (women, aged 15- 55 years old) with Axillary hyperpigmentation were enrolled and randomly assigned 5%Artocarpus lakoocha Heartwood Extract on one axillary region and 5%Artocarpus lakoocha Heartwood Extract Combined 4%Niacinamide on the contralateral region. Participants were instructed to apply the serum twice a day for 12 weeks with follow-up every 4 weeks. The primary end-point for efficacy evaluation was based on Mexameter mean melanin index score, the subject satisfaction, side effects and photography evaluated as the secondary end-point.

Results: 20 subjects completed the study. The average participant age was 36.10 ± 11.02 years old. The results revealed mexameter mean melanin index score, in 5%Artocarpus lakoocha Heartwood Extract Combined 4%Niacinamide and 5%Artocarpus lakoocha Heartwood Extract alone were significantly decrease mean melanin index score from the 4th week of study till the final week (12th week) compare with before treatment but 5%Artocarpus lakoocha Heartwood Extract Combined 4%Niacinamide was not significantly decrease mean melanin index score compare with 5%Artocarpus lakoocha Heartwood Extract alone in all time study. In addition, the participants were satisfied good to excellent response in both the treatment at 12th week. One participant has adverse reaction was found mild symptom of allergic contact dermatitis and discontinuous from this study.

Conclusion: 5%Artocarpus lakoocha Heartwood Extract Combined 4%Niacinamide not significantly better outcomes (mean melanin index score) compared to 5%Artocarpus lakoocha Heartwood Extract alone in all time study treatment. One participant has adverse reaction was found mild symptom of allergic contact dermatitis . Such reason suggested that 5%Artocarpus lakoocha Heartwood Extract Combined 4%Niacinamide not significantly superior to 5%Artocarpus lakoocha Heartwood Extract alone at 12th week.

Keywords: Artocarpus lakoocha Heartwood Extract/Niacinamide /axillary hyperpigmentation/Topical/serum

Introduction

Axillary hyperpigmentation is a frequent cause of cosmetic consultations in women. Postinflammatoryhyperpigmentation(PIH) is one of the most common cause axillary hyperpigmentation. PIH resulting from an increase in melanin production or an abnormal distribution of the pigment in the epidermis and/or dermis after external injury.

The precipitating factor could be related to continuous irritation due to hair removal (shaving, plucking, waxing), cleansing, tight clothes, dry skin, use antiperspirant or deodorant.

Treatment of PIH difficult and prolong time. Protection and treatment of underlying inflammation conditions are the first step.

Artocarpus lakoocha Heartwood Extract have many benefit agent. Oxyresveratrol is one of the important agent that decrease melanin production by tyrosinase inhibitor enzyme. Decreasing of melanin production result to lightening on the skin.

Niacinamide is one derivative of niacin(vitaminB3). Niacinamide is an anti-inflammation agent with depigmenting effect through inhibit transfer of melanosomes from the melanocyte to keratinocyte.

The study of Pichsinee Suwannarat of school of anti-aging and regenerative medicine,2012 found 5%Artocarpus lakoocha Heartwood Extract significantly better out come in decreasing mean melanin index score compare with placebo.

The study of department of Dermatology University of Arkansas for medical sciences,USA found 4%Niacinamide significantly better out come in decrease colorimetic and decrease melanin in histological assessment compare with placebo.

In this study was to assess the efficacy of 5%Artocarpus lakoocha Heartwood Extract Combined 4%Niacinamide Compare with 5%Artocarpus lakoocha Heartwood Extract alone in the Treatment of Axillary hyperpigmentation.

Objective

The purpose of this study was to assess the efficacy of 5%Artocarpus lakoocha Heartwood Extract Combined 4%Niacinamide Compare with 5%Artocarpus lakoocha Heartwood Extract alone in the Treatment of Axillary hyperpigmentation in Thai women.

Research methodology

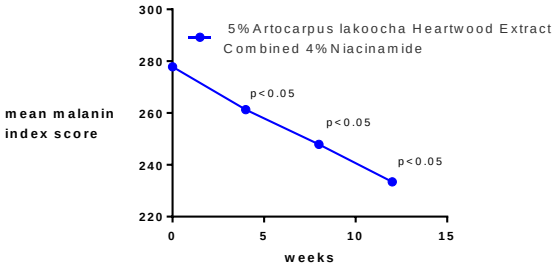
Twenty-one participants (women, aged 15- 55 years old) were enrolled. After obtaining their consent, the eligible participants were randomly assigned through a computer-generated randomization scheme, to 5%Artocarpus lakoocha Heartwood Extract Combined 4%Niacinamide serum apply twice daily on one axillary area and 5%Artocarpus lakoocha Heartwood Extract serum apply twice daily on the contralateral area and for 12 continuous weeks with follow-up interval of every 4 weeks. The primary end-point for efficacy evaluation was based on mexameter mean melanin index score, the subject satisfaction, side effects and photography evaluated as the secondary end-point. The area of measurement are four quadrants of axillary.

Statistical Analysis

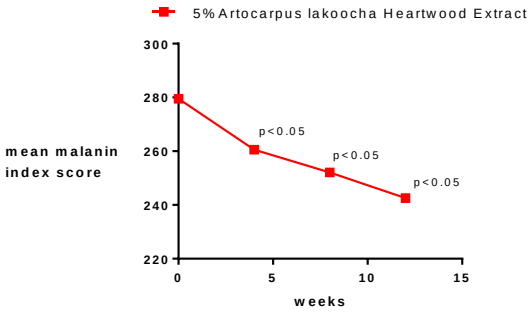
Volunteers' research profile data used descriptive statistical analysis to provide descriptive information. Comparison of mean melanin index score, paired difference between both sides and evaluation of the treatment by dermatologists use Mann Whitney U test or Wilcoxon Match Pair sign rank test. Patient satisfaction at 12th week between both sides and complication use descriptive statistical analysis, the researcher did the following at significance levels of *p-value* <0.05

Results

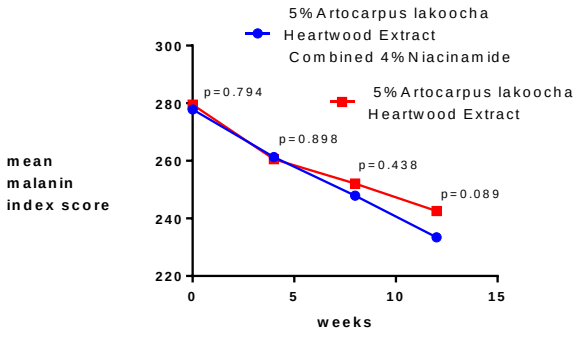
Among the 21 enrolled participants, 20 subjects completed the study. The subjects were female. The average subject age was 36.10 ± 11.02 years old. The results revealed mexameter mean melanin index score, in 5%Artocarpus lakoocha Heartwood Extract Combined 4%Niacinamide and 5%Artocarpus lakoocha Heartwood Extract alone were significantly decrease mean melanin index score from the 4th week of study till the final 12th week(picture1,2) compare before treatment but 5%Artocarpus lakoocha Heartwood Extract Combined 4%Niacinamide was not significantly decrease mean melanin index score compare with 5%Artocarpus lakoocha Heartwood Extract alone in all time study(picture3). In addition, the participants were satisfied good to excellent response in the both treatment at 12th week(picture4) and the photography showed after treatment of skin is whitening than before treatment(picture5). One participant has adverse reaction was found mild symptom of allergic contact dermatitis and discontinuous from this study.



Picture1 Linear graphs of mean melanin index scores in all subjects by use 5% Artocarpus lakoocha Heartwood Extract Combined 4%Niacinamide



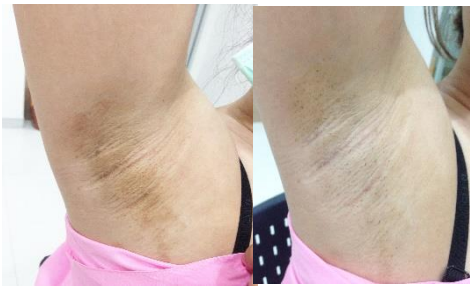
Picture2 Linear graphs of mean melanin index scores in all subjects by use 5%Artocarpus lakoocha Heartwood Extract



Picture3 Linear graphs compared difference mean melanin index score between 5%Artocarpus lakoocha

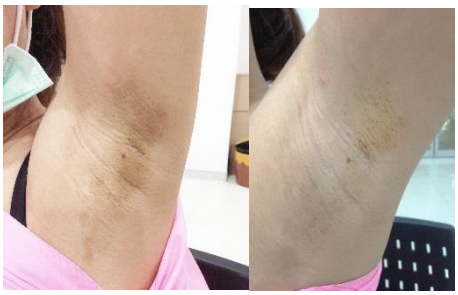
Heartwood Extract Combined 4%Niacinamide and 5%Artocarpus lakoocha Heartwood Extract alone

5%Artocarpus lakoocha Heartwood Extract combined 4%niacinamide



Before treatment at 12th week

5%Artocarpus lakoocha Heartwood Extract



Before treatment at 12th week

Picture 5 There is improvement of axillary hyperpigmentation after treatment of 5%Artocarpus lakoocha Heartwood Extract Combined 4%Niacinamide and 5%Artocarpus lakoocha Heartwood Extract alone compared at 12th week and baseline.

Discussion

Axillary hyperpigmentation is a frequent cause of cosmetic consultations in women. The common treatment are laser, whitening agent, chemical peel, etc. but There is no widely accepted treatment and its limitation of clinical trial.

In 2012th year, The study of Pichsinee Suwannarat of school of anti-aging and regenerative medicine use 5%Artocarpus lakoocha Heartwood Extract that have contain Oxyresveratrol. Oxyresveratrol is one of the important agent that decrease melanin production by tyrosinase inhibitor enzyme and decreasing of melanin production. The result of Pichsinee study showed 5%Artocarpus lakoocha Heartwood Extract significantly better out come in decreasing mean melanin index score compare with placebo from the 4th week of study till the final 12th week and this study showed 5%Artocarpus lakoocha Heartwood Extract alone were significantly decrease mean melanin index score from the 4th week of study till the final 12th week compare before treatment. The conclusion, this study related result with Pichsinee study. The other research about treatment axillary hyperpigmentation is the study of department of Dermatology University of Arkansas for medical sciences,USA used 4%Niacinamide that is an anti-inflammation agent with depigmenting effect through inhibit transfer of melanosomes from the melanocyte to keratinocyte and founded 4%Niacinamide significantly better out come in decrease colorimetic and decrease melanin in histological assessment compare with placebo.

This study have a new idea that combine 2 mechanism of depigmenting effect by used 5%Artocarpus lakoocha Heartwood Extract that decrease melanin production by tyrosinase inhibitor enzyme and used 4%Niacinamide that is a depigmenting effect by inhibit transfer of melanosomes from the melanocyte to keratinocyte. The new idea lead to a hypothesis that 5%Artocarpus lakoocha Heartwood Extract Combined 4%Niacinamide is superior depigmenting effect than 5%Artocarpus lakoocha Heartwood Extract alone in the Treatment of Axillary hyperpigmentation.

The result from this study showed 5%Artocarpus lakoocha Heartwood Extract Combined 4%Niacinamide and 5%Artocarpus lakoocha Heartwood Extract alone were significantly decrease mean melanin index score from the 4th week of study till the final 12th week compare before treatment that conform to in previous study but 5%Artocarpus lakoocha Heartwood Extract Combined 4%Niacinamide was not significantly decrease mean melanin index score compare with 5%Artocarpus lakoocha Heartwood Extract alone in all time study. however if time of study more than 12 weeks, the study to be possible significantly decrease mean melanin index score between 5%Artocarpus lakoocha Heartwood Extract combine 4%niacinamide and 5%Artocarpus lakoocha Heartwood Extract alone because of the linear graphs compared difference mean melanin index score between 5%Artocarpus lakoocha Heartwood Extract Combined 4%Niacinamide and 5%Artocarpus lakoocha Heartwood Extract alone at 12th week that a p-value is 0.089 and close up 0.05 of p-value. Increasing of percentage concentration of niacinamide to be possible significantly decrease mean melanin index score between 5%Artocarpus lakoocha Heartwood Extract combine 4%niacinamide and 5%Artocarpus lakoocha Heartwood Extract alone before 12th week.

Conclusion

5%Artocarpus lakoocha Heartwood Extract Combined 4%Niacinamide and 5%Artocarpus lakoocha Heartwood Extract alone were significantly decrease mean melanin index score from the 4th week of study till the final 12th week compare before treatment but 5%Artocarpus lakoocha Heartwood Extract Combined 4%Niacinamide was not significantly decrease mean melanin index score compare with 5%Artocarpus lakoocha Heartwood Extract alone in all time study(12

weeks). 5%Artocarpus lakoocha Heartwood Extract Combined 4%Niacinamide and 5%Artocarpus lakoocha Heartwood Extract alone could be a useful cosmetic product for treatment axillary hyperpigmentation.

Suggestion

We suggest further investigations to elucidate such as histological assessment, time of study more than 12th week and Increasing of percentage concentration of niacinamide.

Reference

American Academy of Dermatology. Skin of color. Retrive January 5,2013 from http://www.add.org/media-resources/stats-and-facts/prevention-and-care/skin-of-color#.UYUej6LTo78

Juan Pablo, Castanedo-Cazares, Gabryela Lárraga-Piñones, Adriana Ehnis-Pérez, Cornelia Fuentes-Ahumada, Cuauhtemoc Oros-Ovalle2,et al. Topical niacinamide 4% and desonide 0.05% for treatment of axillary hyperpigmentation: a randomized, double-blind, placebo-controlled study. Dovepress 2013;6:29-36

Pichsinee Suwannarat. Efficacy of Artocarpus lakoocha Heartwood Extract in the Treatment of Axillary hyperpigmentation; 2013

Alexis AF, Sergay AB, Taylor SC. Common dermatologic disorders in skin of color: a comparative practice survey. Cutis. 2007;80: 387–394.

Papa CM, Kligman AM. The behavior of melanocytes in inflammation. J Invest Dermatol. 1965;45:465–473.

Mottaz JH, Thorne EG, Zelickson AS. Response of the epidermal melanocyte to minor trauma. Arch Dermatol. 1971;104:611–618.

Tomita Y, Maeda K, Tagami H. Melanocyte-stimulating properties of arachidonic acid metabolites: possible role in postinflammatory pigmentation. Pigment Cell Res. 1992;5:357–361.

A Randomized Split-face Double-blind Control Trial of the Efficacy of 1% Chlorella Vulgaris Extract Versus 0.02% Tretinoin on the Treatment of Periorbital Wrinkles

Thanthip Tanchavalit[1] and Sunisa Thaichinda[2*]
[1,2]*school of Anti-Aging and Regenerative Medicine,Mae Fah Luang University, Bangkok, Thailand*
**Corresponding author. E-mail: dr.sunisa.t@hotmail.com*

Abstract

Topical tretinoin has been long time used for antiwrinkle cream but also has a lot of side effects including burning,itching,and redness. Chlorella vulgaris is small green algae containing many phytonutrients including carotenoids,chlorophyll,tocopherol,ubiquinone which have antioxidant effect and less side effects of burning,itching,and redness. However, there is no clinical study that demonstrating the efficacy of 1% chlorella vulgaris extract for wrinkles treatment compare with 0.02% tretinoin. **Objective**: The purpose of this study is to determine the efficacy of 1% chlorella vulgaris extract compared to 0.02%tretinoin on the treatment of periorbital wrinkle. **Materials and Methods**: Thirty participants (men and women, aged 30-60 years old) with periorbital wrinkle were enrolled and randomly assigned to 1% chlorella vulgaris extract on one periorbital region and 0.02% tretinoin on the contralateral region. Participants were instructed to apply the cream once daily in the evening for 12 weeks with follow-up every 4 weeks. The efficacy evaluation was based on the visioscan result scale, overall improvement score by two physicians, patient satisfaction score, and side effects. **Results**: Thirty participants completed the study. Periorbital wrinkles were significantly improve in week 4th in both 1%chlorella vulgaris and 0.02%tretinoin, measurement by visioscan(p-value<0.001). However, there's no significant difference in improvement of periorbital wrinkle between 1%chlorella vulgaris and 0.02%tretinoin at the end of study(P-value 0.623). For patient satisfaction score 1%chlorella vulgaris was superior to 0.02%tretinoin at p-value 0.025. There was no side effect in chlorella vulgaris group but found 10 cases of side effect(3 cases with erythema and 7 cases with burning sensation around periorbital area) in 0.02%tretinoin. **Conclusion**: 1%chlorella vulgaris is also effective in treatment of periorbital wrinkles with less side effect and more satisfaction to use compare to 0.02%tretinoin

Keywords: periorbital wrinkles, chlorella vulgaris, tretinoin

Introduction

Skin aging is an inevitable process. Skin wrinkle is also sign of skin aging especially periorbital wrinkles. Periorbital wrinkles are easily to be seen and can cause anxiety. Tretinoin cream has been used for periorbital wrinkle treatment for a long time but also has side effects including burning, itching, and redness.

Chlorella vulgaris is small green algae which contain many phytonutrients including carotenoids, chlorophyll, tocopherol, ubiquinone which have antioxidant effect and less side effects of burning, itching, and redness. There is the study of Makpol et. al, showed that 1% chlorella vulgaris extract can increased collagen type1, collagen type3 and elastin in fibroblast.Also in in vitro study of fibroblast exposed to oxidative stress by Makpol, the group of fibroblast treated by chlorella vulgaris has less DNA destruction.

However, there is no clinical study that demonstrating the efficacy of 1% chlorella vulgaris extract for periorbital wrinkles treatment compare with 0.02% tretinoin.

Materials and Methods

Objective

The purpose of this study is to determine the efficacy of 1% chlorella vulgaris extract compared to 0.02%tretinoin on the treatment of periorbital wrinkle.

Research methodology

Thirty participants (men and women, aged 30-60 years old) with periobital wrinkles were enrolled. After obtaining their consent, the eligible participants were

randomly assigned through a computer-generated randomization scheme, to 1% chlorella vulgaris extract once daily in the evening on one periorbital area and 0.02% tretinoin on the contralateral area and for 12 continuous weeks with follow-up interval of every 4 weeks. The efficacy evaluation was based on the visioscan result scale, overall improvement score by two physicians from photographic, patient satisfaction score, and side effects. The area of measurement are point A and point B. Point A is the area lateral to lateral canthus 1.5 centimeters. And point B is the area just below to midpupillary line 1.5 centimeters, respectively.

Statistical Analysis

Volunteers' research profile data used descriptive statistical analysis to provide descriptive information, such as percentages, means, modes, medians, ranges, standard deviations. Comparison of mean visioscan result scale and overall improvement score, paired difference between both sides and evaluation of the treatment by 2 independent dermatologists use Mann Whitney U test or Wilcoxon Match Pair sign rank test. Patient satisfaction at 12th week between both sides and complication use descriptive statistical analysis, the researcher did the following at significance levels of p-value <0.05

Result

Among thirty enrolled participants,30 subjects completed the study. The subject were female (27/30,90%) and male (3/30,10%). The average subject age was 42.07 ± 10.85 years old, start from 30 years old to 60 years old.

30 subjects completed the 12-week period study. By using visioscan as the measurement, the result revealed that wrinkles in periorbital areas were significantly improved in week 4th in both 1%chlorella vulgaris group and 0.02%tretinoin at p-value 0.001. However, in comparison between 1%chlorella vulgaris and 0.02%tretinoin at the 12-week period study shown no significant difference in improvement of periorbital wrinkle as shown in table1.

Table 1. Visioscan result scale between before treatment (week0) and after treatment (week12) Mean±SD

Visioscan result scale for wrinkle	1% chlorella vulgaris (n=30)	0.02% Tretinoin (n=30)	t	P-value
Point A	6.03±2.53	6.25±1.91	-0.382	0.704
Point B	7.40±2.16	7.17±1.42	0.482	0.632
Mean	6.88±3.92	7.38±3.93	-0.494	0.623

*No significant difference using Independent-Sample T-test

Evaluation with overall improvement score by two physicians who were not association in this research using photographics comparison between week 0th and week 12th revealed that no significant difference between 1%chlorella vulgaris extract and 0.02%tretinoin as shown in table2.

Table 2. Overall improvement score between before treatment (week0) and after treatment (week12) evaluated by 2 physicians

	1% chlorella vulgaris (n=30)	0.02% Tretinoin (n=30)	P-value
1st physician	1.53±0.57	1.53±0.63	0.907
2nd physician	1.37±0.72	1.4±0.56	0.954
mean	1.45±0.58	1.47±0.54	0.938

For patient satisfaction score 1% chlorella vulgaris extract was superior to 0.02%tretinoin at P-value 0.025 as shown in table3. This may be due to side effect of 0.02%tretinoin which was redness (3subjects,10%) and burning sensation (7subjects,23.3%) while in 1% chlorella vulgaris was not shown any side effect among subjects.

	1% chlorella vulgaris (n=30)	0.02% Tretinoin (n=30)	P-value
Patient satisfaction score	2.03±0.61	1.67±0.61	0.025*

Table3.Patient satisfaction score

*significant difference by using Mann-Whitney U-test

Discussion

From the result in 1%chlorella vulgaris group shown improvement of periorbital wrinkle since 4th week with p-value<0.001 consistent with previous study of Makpol et al.(Makpol et al.,2009) shown improvement of wrinkle in 28 days.

In 0.02%tretinoin group also shown improvement of periorbital wrinkle at 4th week with p-value<0.001 consistent with previous study of Kafi R. et al. (Kafi R, Kwak HS, Schumacher WE, et al. 2007) that shown improvement after 4th-8th week after tretinoin use.

Participants in both group have shown clinical improvement in periorbital wrinkle with no significant difference in statistics at week 12th. But in 0.02%tretinoin group, there was 10 participants report side effect of the cream at 2nd - 4th week(redness 3 cases in 2nd week and redness with burning sensation 7 cases in 4th week). For patient satisfaction score in 1%chlorella vulgaris group was superior to 0.02%tretinoin group at P-value 0.025. This maybe due to less side effect of 1%chlorella vulgaris.

From all the result we found that 1%chlorella vulgaris has the same efficacy in treatment of periorbital wrinkle compare to gold standard,0.02% tretinoin. But 1%chlorella vulgaris extract was superior to 0.02%tretinoin in patient satisfaction score with less side effect compare to 0.02%tretinoin.

So we assume that 1%chlorella vulgaris is another good choice for periorbital wrinkle treatment due to equal efficacy to gold standard,0.02%tretinoin, but more comfortable to use due to no side effect.

Conclusion

1%chlorella vulgaris could accomplish equal efficacy in clinical improvement of periorbital wrinkles compare to gold standard,0.02% tretinoin since 4th week with no recorded side effect. Such reason suggested that 1%chlorella vulgaris is also effective for periorbital wrinkle treatment.

Suggestion

We suggest further investigations to elucidate whether a longer period of study on 1%chlorella vulgaris effect after off-treatment, comparison more concentration of chlorella vulgaris extract with gold standard medicine.

Reference

Cheng FC, Lin A, Feng JJ, Mizoguchi T, Takekoshi H, Kubota H, Kato Y, Naok Y. Effects of chlorella on activities of protein tyrosinase phosphatases, matrix metalloproteinases, caspases, cytokine release, B and T cell proliferations, and phorbol ester receptor binding. J. Med. Food.2004. 7,146-152

Cherng JY, Liu C, Shen CR, Lin HH, Shih MF.Beneficial effects of chlorella-11 peptide on blocking the LPS-induced macrophage activation and alleviating the thermal injury induced inflammation in rats. Int. J. Immunopathol. Pharmacol. 2010.23,811-820

Chiu-Lan Chen, Shu-Fen Liou, Su-Jong Chen, Mei-Fen Shih. Protective effects of chlorella-derived peptide on UVB-induced production of MMP-1 and degradation of procollagen genes in human skin fibroblasts. Regulatory toxicology and pharmacology. 2011. 60,112-119

Kafi R, Kwak HS, Schumacher WE, et al. Improvement of naturally aged skin with vitaminA(retinol). Arch Dermatol. 2007; 143-606.

Fisher GJ, Wang ZQ,Datta SC, et al. Pathophysiology of premature skin aging. Trans NY Acad Sci.1969;31:992

Ichioka M, Endo H. Effect of light on cellular carotenoids formation in chlorella regularis S-5o grown on glucose. Annual report of the Yakult Institute of Microbiology Research. 1973;91-99

Makpol et al. Chlorella vulgaris modulates hydrogen peroxide-induced DNA damage and telomere shortening of human fibroblasts from different aged individuals. Afr. J. Trad. CAM. 2009. 6(4):560-572

Makpol et al. Comparative effect of piper betle, chlorella vulgaris and tocopherol-rich fraction on antioxidant enzymes activity in cellular ageing of human diploid fibroblasts. BMC Complementary and Alternative Medicine. 2013, 13:210

A Split-face, Double blind, Randomized Clinical Trial the Effectiveness of 3% Lentil Seed Extract (Lens Esculenta) Cream Versus 0.025% Tretinoin (Retinoic acid) Cream for Minimizing Pore Size

Pijiporn Sritongsuk[1*] and Paisal Rummaneethorn[2]

[1]*Student in program Master of Science Program in Dermatology, School of Anti-aging and regenerative medicine,*
[2]*Advisor, School of Anti-aging and regenerative medicine, Mae Fah Luang University, 38/11-13 Asoke Place Building, Sukhumvit 21, Klong Toey Nua, Wattana, Bangkok, 10110, Thailand*
[*]*Corresponding author. E-mail: monkey_oil@hotmail.com*

Abstract

An excess of sebum secretion cause pores to widen. Topical retinoid is a standard treatment for photoaging and skin roughness which reduce sebum production and increase collagen production, therefore improvement of pore, but its adverse effects limit their acceptance by patients. 3% lentil seed extract has been studied being effective on reduction of sebum production and refinement of pores.

Keywords: Pore size/ Sebum / Lentil / Tretinoin

Introduction

Enlarge pore size are common found in clinical practice, the result from excess sebum secretion from intrinsic and extrinsic factor, It is usually found in oily and combination skin, common on nasal and cheek area. That is the cause of skin roughness. Nowadays treatments have invented for this condition.

Topical retinoid is a standard treatment for photoaging and skin roughness which reduce sebum production and increase collagen production, therefore improvement of pore. However its adverse effects such as redness, irritant reaction and scaling and these limit their acceptance by patients, lead to discontinue used.

Lens esculenta is the source of Oligosaccharide (P-Refinyl), for refinement of pores which limits the sebum production, stimulates the expression of collagen I which reinforcing pore wall support structure and restores a normal keratinization process and reduces the number of nucleated keratinocytes which rigidify the pore wall and prevent its closing.

In this research, the study had compared treatment of pore size between 3% lentil seed extract cream and 0.025 % tretinoin cream. And evaluation by comparing its efficacy from photographs by digital camera, average roughness (R2) by Visioscan® VC 98, sebum secretion by Sebumeter® , side effects and volunteers' satisfaction.

Objective

To compare the efficacy of a 3% lentil seed extract cream with 0.025 % tretinoin cream for treatment of enlarged pore size

Materials and Methods

Research methodology

30 volunteers (age range 25-55 years) with enlarged pore size on nasal and cheek area were randomly applied 3% lentil seed extract cream on nasal and cheek area twice a day and 0.025 % tretinoin cream on the contralateral sides once a day for 8 weeks with follow-up every 2 weeks, The pore sizes were evaluated average roughness (R2) by Visioscan ® VC 98, and the measurement of sebum secretion by Sebumeter ®. Both values and adverse effects were assessed at the start of the study and the following in every 2 weeks. Side effects and volunteers' satisfaction were assessed by questionnaires.

Statistic for Data Analysis

Volunteers' research profile data using descriptive statistical analysis to provide descriptive information, such as percentage, means, modes, medians and standard deviations. Comparison of mean of average roughness (R2) and mean sebum secretion between The 3% lentil seed extract cream and 0.025 % tretinoin cream use Mann-Whitney U-test. Comparison of mean of average roughness (R2) and mean sebum secretion between baseline, 2th, 4th, 6th and 8th week uses Wilcoxon sign rank test. Comparison of improvement evaluated by patients uses Wilcoxon sign rank test. Comparison of satisfaction evaluated by patients use Wilcoxon sign rank test. The side effects of both drugs use descriptive statistical analysis to provide descriptive information. The researcher did the following at significance of p-value < 0.05

Results

Thirty volunteers completed the study. The study found that The 3% lentil seed extract cream and 0.025 % tretinoin cream statistically significant difference ($p<0.001$) of skin roughness when evaluated by comparing mean average roughness (R2) at week 8 in each groups, but comparing between two groups was no statistically significant difference ($p=0.79$) (e.g. Figure 1). And evaluated by comparing mean sebum secretion resulted in statistically significant difference ($p<0.001$) of sebum secretion at week 8 in each groups, but comparing between two groups was no statistically significant difference ($p=0.11$) (e.g. Figure 2). The volunteers were significantly more adverse effects with 0.025 % tretinoin cream than 3% lentil seed extract cream. These were generally well tolerated and no subject discontinued participating in the study because of side effects. For satisfactory score was no statistically significant difference between two groups.

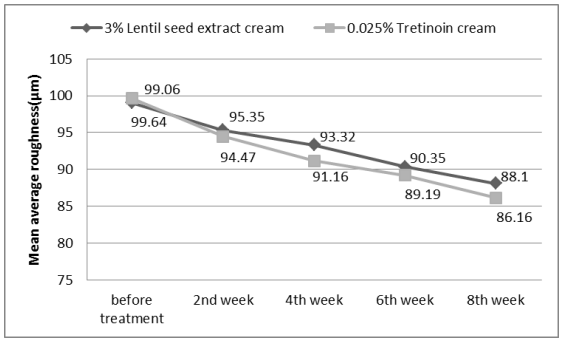


Figure 1 Linear graphs compared difference mean average roughness (µm) Between 3% lentil seed extract cream and 0.025 % tretinoin cream.

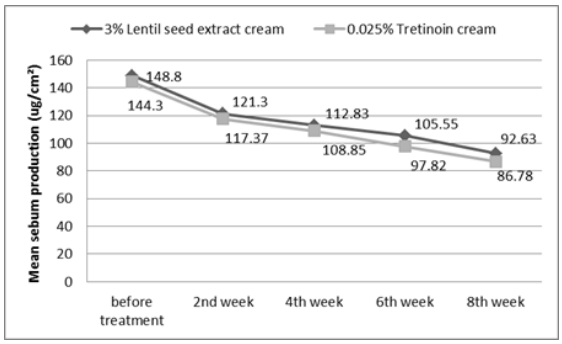
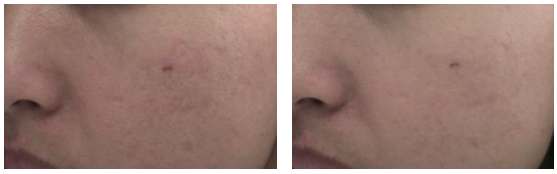


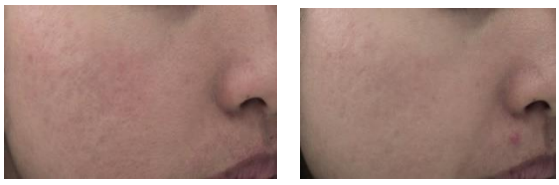
Figure 2 Linear graphs compared difference mean sebum secretion (µg/cm²) Between 3% lentil seed extract cream and 0.025 % tretinoin cream.

3% lentil seed extract cream



Before **After 8 weeks**

0.025 % tretinoin cream



Before **After 8 weeks**

Figure 3 To compare photographs of digital camera between the treatments by 3% lentil seed extract cream and 0.025 % tretinoin cream in 8th week and before treatment.

Discussion

From the research, we found that the volunteers in the group applied 3% lentil seed extract cream and the group applied 0.025 % tretinoin cream, both have been shown statistically significant difference in improvement of skin roughness which was conformed with research in the past. They found that 3% lentil seed extract is statistically significant effective in treating enlarge pore size by reduce sebum secretion result in improve skin roughness and refinement of pore.

The research outcome that the 3% lentil seed extract cream and 0.025 % tretinoin cream statistically significant difference when evaluated by comparing mean average roughness (R2) at week 8 in each groups, but comparing between two groups was no statistically significant difference. And evaluated by comparing mean sebum secretion resulted in statistically significant difference of sebum secretion at week 8 in each groups, comparing between two groups was no statistically significant difference. The volunteers were significantly more adverse effects with 0.025 % tretinoin cream than 3% lentil seed extract cream. These were generally well tolerated and no subject discontinued participating in the study because of side effects.

For satisfactory score was no statistically significantly difference between two groups. Therefore, the research results in efficacy, side effects, and satisfaction in treatment has supported the research assumption as mentioned in the beginning.

Conclusion

3% lentil seed extract cream is equally effective as 0.025 % tretinoin cream for reduce sebum production and improve skin roughness, but 0.025 % tretinoin cream more adverse effects than 3% lentil seed extract cream. Such reason suggested that 3% lentil seed extract cream is effective for enlarged pore size treatment.

Reference

Darlenski, R., Surber, C. & Fluhr, J. W. (2010). Topical retinoids in the management of photodamaged skin: from theory to evidence-based practical approach. Br J Dermatol. 163(6),1157-1165.

Katsuta Y, Iida T, Inomata S & Yoshida S. (2004), Improving the Appearance of Facial Pores, Cosmetic & Toiletries, 119(10),59-64

Nyirady J, Bergfeld W & Ellis C. (2001). Tretinoin cream 0.025% for the treatment of photodamaged facial skin: A review of 2double-blind clinical trials.Cutis,68,135–142.

Roh M, Han M, Kim D & Chung K. (2006) , Sebum output as a factor contributing to the size of facial pores, British J of Dermatology, 155, 890-894.

Sugata K, Nishijima T, Kitahara T & Takema Y. (2008), Confocal laser microscopic imaging of conspicuous facial pores in vivo: relation between the appearance and the internal structure of skin, Skin Research and Technology, 14, 208-212.

Sugiyama-Nakagiri, Y., Sugata, K., Iwamura, M., Ohuchi, A. & Kitahara, T. (2008). Age- related changes in the epidermal architecture around facial pores. J Dermatol Sci., 50(2), 151-154.

Uhoda E, Pierard-Franchimont C, Petit L, Pierard GE. (2005), The conundrum of skin pores in dermocosmetology. Dermatology2005;210(1):3-7

A Split-face Double-blind Randomized of the Efficacy of Topical Momordica Cochinchinensis Compare with 0.02% Tretinoin in the Treadment of Periorbital Wrinkle

Wachiraporn Thaiprayoon[1*] and Tanomkit Pawcsuntorn[2]

[1]*Master of Science Program in Dermatology, School of Anti-Aging and Regenerative Medicine, Mae Fah Luang University, Asoke road, Sukhumvit 21 Klong Toey Nua, Wattana, Bangkok 10110*
[2]*Corresponding author, School of Anti-Aging and Regenerative Medicine, Mae Fah Luang University, Asoke road, Sukhumvit 21 Klong Toey Nua, Wattana, Bangkok 10110*
**E-mail: tukta_064@hotmail.com*

Abstract

The periorbital region serves as a sign of aging because this area was seen easy. 0.02 % Tretinoin is a standard for treatment photoaging but its adverse effects limit their acceptance by patients. *Momordica cochinchinensis* which has an antioxidant effects, antiwrinkles and increase of elasticity of skin. However, the antiwrinkle effect of *Momordica cochinchinensis* compare with standard treatment as 0.02 % tretinoin has not been study.

Objective: To compare the efficacy of a topical *Momordica cochinchinensis* with 0.02 % tretinoin in the treatment of periorbital wrinkles

Materials and Methods: 30 volunteers, who has grade II-IV (Rao-Goldman 5 point Visual Scoring Scale) periorbital wrinkles, aged 30-60 years old were asked to apply 1% topical *Momordica cochinchinensis* and 0.02% tretinoin around the right or left (randomly determined by the computer system) periorbital region , twice daily for 8 weeks. The wrinkles were evaluated by Visioscan ® VC 98 , and the measurement of skin elasticity using Cutometer ® MPA 580. Both values and adverse effects were performed at the start of the study and the following 4, 6 and 8 weeks. The patient's satisfaction and the clinical improvement by 3 doctors who not involved in this study were evaluated at week 8.

Results: The results from visioscan showed the significant improvement of the under eyes wrinkles at week 8 in both topical *Momordica cochinchinensis* and 0.02% tretinoin , comparing between two groups was no significantly difference. Statistically, topical *Momordica cochinchinensis* and 0.02% tretinoin resulted in significant increase of elasticity of lateral canthal wrinkles and below eye wrinkles at week 8 in both groups, comparing between two groups was no significantly difference. The clinical improvement showed minimal improvement of lateral canthal wrinkles and below eye wrinkles in both groups. The volunteers were significantly more satisfied with topical *Momordica cochinchinensis* than 0.02% tretinoin. This study found 3 volunteers had adverse effects of scales at side treated with 0.02% tretinoin.

Conclusion: The topical *Momordica cochinchinensis* was as much as efficacy as 0.02% tretinoin in the treatment of periorbital wrinkles with no recorded adverse effect.

Keywords Periorbital Wrinkles/Skin elasticity/Topical *Momordica cochinchinensis* /Topical Tretinoin

Introduction

The periorbital region serves as a sign of aging because this area was seen easy. Periorbital wrinkles are the result from intrinsic aging and extrinsic aging. Nowadays treatments have invented for this condition [3].

0.02 % Tretinoin is a standard treatment and now recognized by the FDA for treatment photoaging but its adverse effects such as redness, irritant reaction and scaling and these limit their acceptance by patients, lead to discontinue used [2]. *Momordica cochinchinensis* is the source of antioxidants such as carotenoids (beta-carotene and lycopene) and vitamin E, thus it can prevents collagen degradation, promotes new collagen regeneration in the dermis, resulting in the reduction of wrinkles and the increase of elasticity of skin [4]. *Momordica cochinchinensis* has been studied being effective on reduction of wrinkles and the increase of elasticity of skin because its potent antioxidant effects [1],[5]. But these studies were the comparison of the efficacy of *Momordica cochinchinesis* with placebo, However, the antiwrinkle effect of *Momordica cochinchinesis* compare with standard treatment as 0.02 % tretinoin

has not been study before, thus this is the inspiration of my study.

In this research, the study had compared periorbital wrinkle treatment by applying topical Momordica cochinchinesis and 0.02% tretinoin by comparing its efficacy from visioscan scores measured by Visioscan ® VC 98, elasticity scores measured by Cutometer ® MPA 580, the clinical improvement by 3 doctors who not involved in this study, which evaluated from the photographs of digital camera, comparing adverse effects and volunteers' satisfaction.

Objective

To compare the efficacy of a topical *Momordica cochinchinensis* with 0.02% tretinoin in the treatment of periorbital wrinkles.

Materials and Methods

30 volunteers with grade II-IV (Rao-Goldman 5 point Visual Scoring Scale) periorbital wrinkles, aged 30-60 years old were asked to apply 1% topical Momordica cochinchinensis and 0.02% tretinoin around the right or left periorbital region (dose = 0.2 mg/side) which assigned through a computer-generated randomization scheme, twice daily for 8 weeks. The area of measurement are point 1 and point 2. Point 1 is the area lateral to lateral canthus 1.5 centimeters and point 2 is the area below to lateral canthus 1.0 centimeters. The wrinkles were evaluated by Visioscan ® VC 98 and the measurement of skin elasticity using Cutometer ® MPA 580. Both values and adverse effects were performed at the start of the study and the following 4, 6 and 8 weeks. The patient's satisfaction and the clinical improvement by 3 doctors who not involved in this study were evaluated at week 8.

Statistical Analysis

Volunteers's research data used descriptive statistical analysis to performed descriptive information, such as means, medians, standard deviations and percentages. Comparison of the means of wrinkles and skin elasticity in between groups use Repeated measure ANOVA and compare between visits use Multiple comparison, Bonferoni method. The patient's satisfaction at week 8 use Wilcoxon Match Pair sign rank test and the clinical improvement by 3 doctors use Mann Whitney U test. McNemar test use for adverse effects and the researcher did the following at significant levels of p-value < 0.05.

Results

The topical *Momordica cochinchinensis* applied-side was equal the other side of 0.02% tretinoin in the efficacy for treatment of periorbital wrinkles that improved significantly at week 8. The results from visioscan showed the significant improvement of the under eyes wrinkles at week 8 in both topical *Momordica cochinchinensis* and 0.02% tretinoin (p-value = 0.005), comparing between two groups was no significantly difference (p-value = 0.501). Statistically, topical *Momordica cochinchinensis* and 0.02% tretinoin resulted in significant increase of elasticity of lateral canthal wrinkles and below eye wrinkles at week 8 in both groups (p-value = 0.002 and 0.008 respectively) , comparing between two groups was no significantly difference (p-value = 0.981 and 0.349 respectively). The clinical improvement by 3 doctors evaluation showed minimal improvement of lateral canthal wrinkles and below eye wrinkles in both groups. No significantly difference between two groups (p-value = 0.765 and 0.526 respectively). The satisfied scores were significant difference on lateral canthal wrinkles and below eye wrinkles treated with topical *Momordica cochinchinensis* better than 0.02% tretinoin (p-value = 0.033 and 0.016 respectively). The adverse effects revealed no differ significantly between two groups (p-value = 0.250).

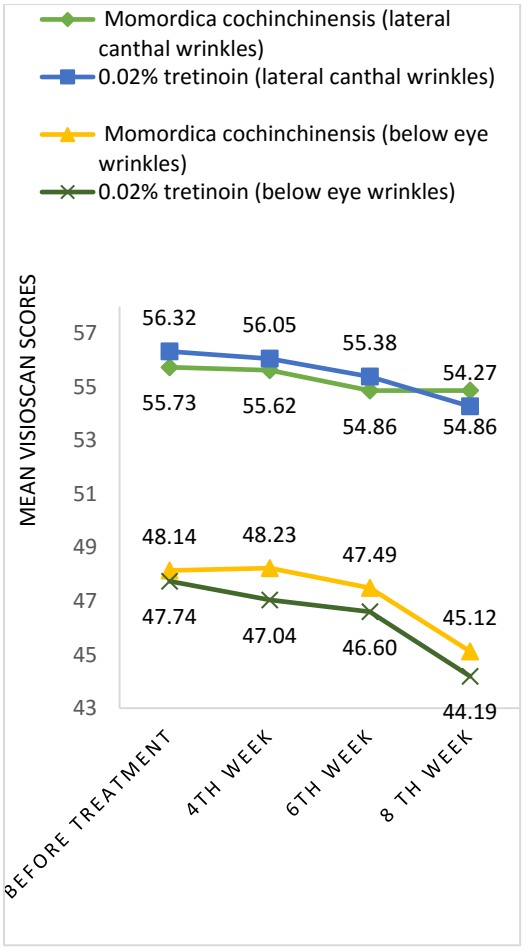


Figure 1 Linear graphs compared difference mean change of visioscan scores in all subjects at point 1 and point 2

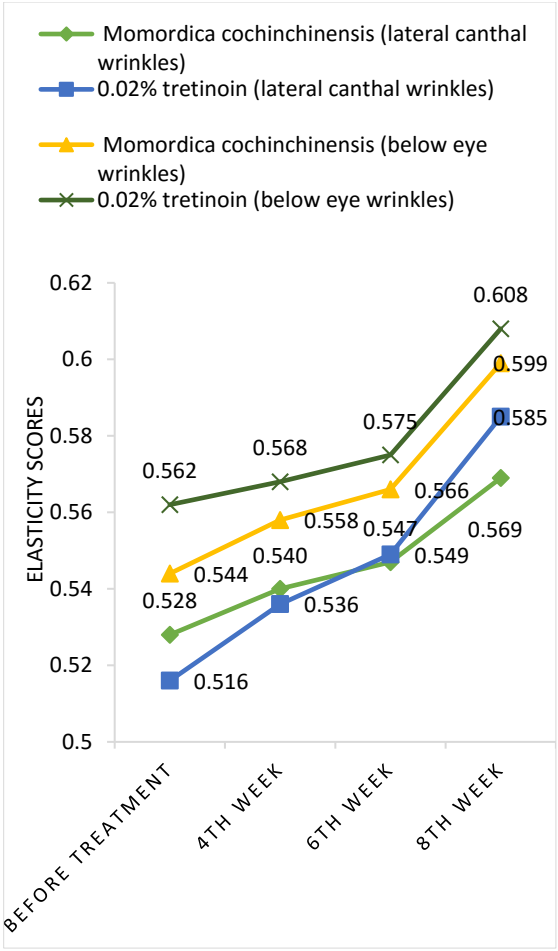
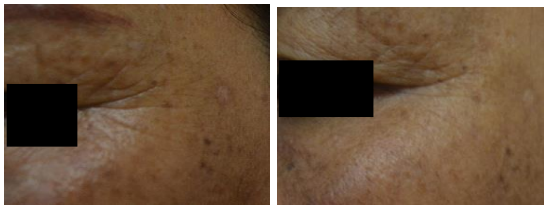
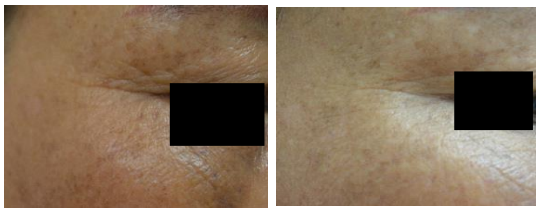


Figure 2 Linear graphs compared difference mean change of elasticity scores in all subjects at point 1 and point 2



Before After 8 weeks

1% topical *Momordica cochinchinesis*



Before After 8 weeks

0.02% tretinoin

Figure 3 There is improvement of wrinkle after treatment of this example subject compared in 8th week and before treatment.

Discussion

Volunteers in the group applied topical *Momordica cochinchinensis* and the group applied 0.02% tretinoin have shown significantly mean improvement in parameters of wrinkles at under eyes wrinkles at week 8, comparing between two groups was no significantly difference. But wrinkles at lateral to lateral canthus were not improvement, because maybe this area always move in static and dynamic movement , thus this is a difficult area to treat and maybe must use more duration of treatment for see the improvement of results. Statistically, topical *Momordica cochinchinensis* and 0.02% tretinoin resulted in significant increase of elasticity of lateral canthal wrinkles and below eye wrinkles at week 8 in both groups, comparing between two groups was no significantly difference. This study supports previous studies [1],[5].

Momordica cochinchinensis helps improvement of wrinkles and increase of elasticity of skin because it is the source of antioxidants such as carotenoids (beta-carotene and lycopene) and vitamin E, thus it can prevents collagen degradation, promotes new collagen regeneration in the dermis, resulting in the reduction of wrinkles and the *increase* of elasticity of skin [4].

This study found 3 volunteers had adverse effects of scales at side treated with 0.02% tretinoin, this is the common adverse effect from tretinoin, whereas we did not find any adverse effects form *Momordica cochinchinensis.* However this revealed no differ significantly between two groups.

The satisfied scores were significant difference on topical *Momordica cochinchinensis* better than 0.02% tretinoin ,because *Momordica cochinchinensis* showed no adverse effect.

Conclusion

The topical *Momordica cochinchinensis* was as much as efficacy as 0.02% tretinoin in the treatment of periorbital wrinkles with no recorded adverse effect and could be a useful functional cosmetic product.

References

1. Natthinee Nantalit, Surapol Natakankitkul, anee Sirisa-ard & Suporn Charumanee.(2011). Development of anti-wrinkle cosmetic from aril oil of Momordica ochinchinensis (Lour.) Spreng in nanostructure lipid carriers (NLC). Thesis. Master of Science (Pharmaceutical Sciences). Chiang Mai University, Chiang Mai.
2. Nyirady J, Bergfeld W & Ellis C. (2001). Tretinoin cream 0.02% for the treatment of photodamaged facial skin: A review of 2 double-blind clinical trials. Cutis, 68,135–142.
3. Rhesa May P, Manaloto & Tina S. Alster. (1999). Periorbital rejuvenation: A review of dermatologic treatments. Dermatol Surg, 25, 1-9.
4. Vuong, L.T., Dueker, S.R., Murphy & S.P. (2002). Plasma beta-carotene and retinol concentrations of children increase after a 30-d supplementation with fruit Momordica cochinchinensis (gac). American Journal of Clinical Nutrition, 75(5), 872-879.
5. Wiphattra Suphachinda, Panvipa Krisdaphong, Myuree Kalayawattanakul. (2008). The development and clinical evaluation of standardized gac fruit extract. Thesis. Master of science (Coematic Science). Mae Fah Luang University, Chiang rai

A Split-face, Randomized, Double-blind Clinical Trial for Eyebrow Lifting Comparing between Intradermal Onabotulinum Toxin and Abobotulinum Toxin Injection

Kanwara Sukkho,MD.[1] and Paisal Rummaneethorn MD.[2]

[1,2] *School of Anti-aging and Regenerative Medicine,Mae Fah Luang University,Asoke place,Bangkok,10110 and Thailand*
E-mail : kwr116@gmail.com

Abstract

Background An intradermal botulinum toxin injection is a technique of microinjections of diluted botulinum toxin to preserve facial mobility in order to preserve natural beauty. And this technique is widely use for facelift. Between Onabotulinum toxin and Abobotulinum toxin which one gives better result in lifting eyebrow with intradermal technique injection. Objective To evaluate the therapeutic result in efficacy and duration of action of intradermal Onabotulinum toxin compared to Abobotulinum toxin injection in the treatment of lifting eyebrow. Materials and methods Ten subjects were injected single treatment with intradermal Onabotulinum toxin and Abobotulinum toxin split-face,randomized,double-blind to lift eyebrow at hairline then all enrolled subjects has been measured mean brow height change and taken photograph by VISIA® before and after injection in each follow up visit. Clinical visits were scheduled at immediate after treatment up to 120 days. Result There were significant difference of brow height at lateral canthus(P=0.021)after day 14 of treatment ,at mid-pupil after day 30 (P=0.01), no significant difference between the length from lateral canthus to hairline (P>0.05) but there were significant difference between the length from lateral canthus to tail of eyebrow(P=0.011)after day 0 of treatment. There was no side effect occurred. Measured brow height at day 120 of follow-up does not decline to baseline. Conclusion In lifting eyebrow with intradermal injection technique,Abobotulinum toxin is more effective than Onabotulinum toxin but both are consider safe with no side effect. The duration of action have to study further more.

Keywords : Onabotulinum toxin,Abobotulinum toxin, intradermal injection, lifting eyebrow

Introduction

Eyebrow lifting procedure, there are 2 options in general(1)Botulinum toxin injection (2) Endoscopic forehead lift technique. Botulinum toxin injection is easier way and non-invasive technique so more popularity gain. Also there are lots of botulinum toxin injection techniques.Intradermal Botulinum toxin injection is a procedure that benefits oily skin and oversized pores. It is not injected into the muscle that target facial expression.Instead, it is injected into the arrector pili cause minimize the pore results in a smaller skin surface. This stretches the skin causes inadvertent facelift. Onabotulinum toxin and Abobotulinum toxin both are type A botulinum toxin.And there are data support their lifting efficacy.But not enough data to compare their efficacy in lifting eyebrow before.For this research,The researcher made an effort to evaluate efficacy and duration of action compared between Onabotulinum toxin and Abobotulinum toxin injection in lifting eyebrow.

Objectives

To evaluate the therapeutic result in efficacy and duration of action of intradermal Onabotulinum toxin compared to Abobotulinum toxin injection in the treatment of lifting eyebrow.

Materials and Methods

A split-face,Randomized,double-blind clinical trial.Ten participants with desire to lift eyebrow, age 25-55 years old, 9 women and 1 man, were randomly assigned to the treatment of lifting eyebrow with intradermal Onabotulinum toxin 12 U and Abobotulinum toxin 30 U at temporal area (with ratio 1:2.5).Each side of hairline at temporal area will be injected 6 points of botulinum toxin (2 rolls,3points each roll,1 cm apart each point).Subjects received single treatment at day0 and adverse events and efficacy measures were assessed at each follow-up visit for 120 days(at days0, 14, 30 ,60, 90,120). Photographic documentation using identical camera setting, subject positioning and environmental light by VISIA® Complexion Analysis System was done before treatment and at each follow-up visit. Brow height measurement was independently calculated by three mask physicians.6 sets of data were collected.(1)brow height, pretreatment at lateral canthus (2)brow height, pretreatment at mid-pupil (3)brow height, posttreatment

at lateral canthus (4)brow height, posttreatment at mid-pupil (5)the length from lateral canthus to hairline (6)the length from lateral canthus to tail of eyebrow. Brow Height treatment satisfaction Questionnaire was rated by subjects ranging from 1 to 7 (1 = very dissatisfied to 7 = very satisfied)

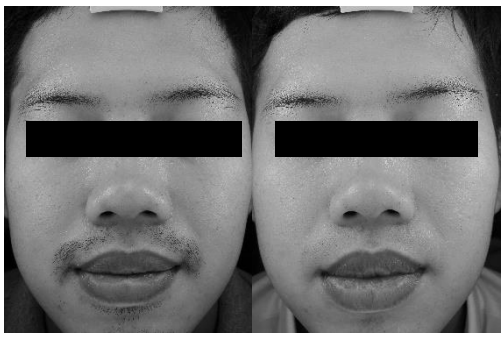
Data analysis

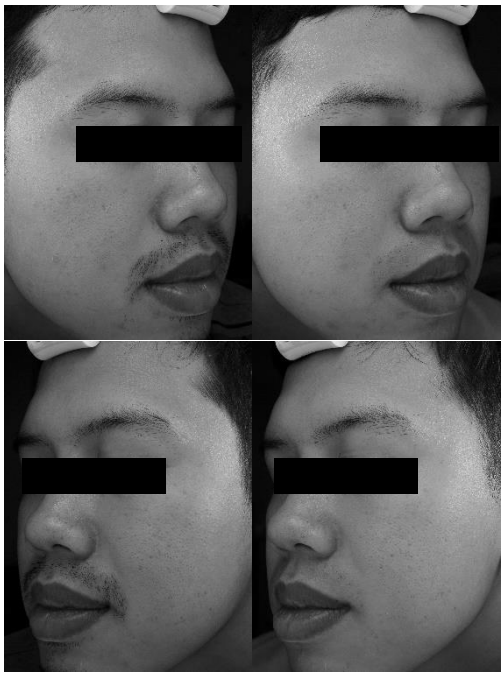
The data has statically analyzed by Wilcoxon Signed Ranked test

Results

All 10 patients complete the study.The result show that there were significant difference of brow height at lateral canthus (P = 0.021) after day 14 of treatment and also significant difference of brow height at mid-pupil after day 30 (P = 0.01) .There were no significant difference between the length from lateral canthus to hairline (P > 0.05) but there were significant difference between the length from lateral canthus to tail of eyebrow (P = 0.011).The difference in mean brow height at mid-pupil achieved after Onabotulinum toxin injection was 0.094 cm. and after Abobotulinum toxin injection was 0.122 cm.The difference in mean brow height at lateral canthus achieved after Abobotulinum toxin injection was 0.105 cm. and after Abobotulinum toxin injection was 0.309 cm.

Brow height treatment satisfaction score = 5.10 (1= very dissatisfied to 7 = very satisfied). There was no side effect after treatment was noted. Time to return to baseline after treatment has to study further more.





Picture 1 before and after treatment (Right eyebrow was injected with Obabotulinum toxin. Left eyebrow was injected with Abobotulinum toxin)

Discussion

As has been trend toward aesthetic surgery nowaday,the newer technique such as botulinum toxin injection has become more popular.More and more than 8 botulinum neurotoxin products has launched to the market.Some products have claimed their ability to lift face better than others product.From prior study intradermal botulinum toxin typeA can lift the face.Onabotulinum toxin and Abobotulinum toxin both are type A toxin so can be injected to lift face,especially midface lifting,The result of this research has confirmed that data. Prior the clinical trial we search for dose equivalence between Onabotulinum toxin and Abobotulinum toxin to use appropriately the ratio is 1:2-1:3 (range 1:2-1:6) so the researcher use 1:2.5 after calculate the quantity of toxin.And doses of botulinum toxin uses for lifting eyebrow from prior research the researcher use 7-10 U of Botulinum toxin A injected at temporal area lateral to orbital rim. so this research use 12 U of Onabotulinum toxin and 30 U of Abobotulinum toxin injection.Eyebrow lifting with botulinum toxin there is one serious side effect that is ptosis,it is not occur very often but quite interrupt daily life activity of the patient.Ptosis cause after botulinum toxin injection because the toxin diffuse to levator palprebrae superioris muscle.If there is a way to lift eyebrow without risk of ptosis should be consider.From prior research the toxin has injected lateral part of Orbicularis oculi muscle,there is still a chance to cause ptosis.So the researcher has conducted this research to avoid ptosis by injected botulinum toxin far from Orbicularis oculi muscle,by injected at the hairline.And our data demonstrate that significant brow elevation is achieved both at mid-

pupillary line after day 30 and at the lateral canthus after day 14.The slow response of mid-pupil is to be expected considering that botulinum toxin was administered to the temporal area further than lateral canthus.A visibly appreciable amount of brow elevation is achieved.No ptosis occurred.Patients undergoing this procedure experienced a low rate of side effect.The most frequent side effect was slightly edema (100%) because intradermal injection technique, but last no longer than 3 hours.No one got bruise, infection ,headache (0%)The total length of follow-up for all patients was 120 days.The observed effect of brow elevation was noted to have persisted in all patients.Although our study did not extend beyond 120days ,we would expect the brow lift effect to demonstrate longer than 180 days.The amount of elevation is not predictable.Therefore, precise changes in brow height cannot be achieved.

In conclusion

Intradermal Abobotulinum toxin injection have higher efficacy in lifting eyebrow more than Onabotulinum toxin but both have appreciable efficacy in lifting eyebrow with no serious side effect.The duration of action has to study further more.

Reference

Trindade De Almeida,Secco,&Carruthers,2011; Yang&Jung,2013

Chariya Petchngaovilai,MD; journal of cosmetic dermatology;2009

Hambleton&Pickett,1994;Hexel et al., 2012; Karsai&Raulin,2009;Wenzel,Jone,&Borrego, 2007

Min S. Ahn,M.D.,Michael Catten,M.D.,and Corey S. Maas, M.D. temporal Brow Lift using Botulinum Toxin A,plastic and reconstructive surgery;2003

Won,C.H.,Lee,H.M.,Lee,W.S.,Kang,H.,Kim,G.J.,Kim ,W.S.,Lee,J.H.,Lee,D.H.&Huh,C.H.(2013)Effi cacy and safety of a novel botulinum toxin type A product ,Dermatol Surg,39

A Randomized, Single-blinded, Side by Side Control Study by The Comparison of Noninvasive Radiofrequency and Intradermal Botulinumtoxin TypeA Injection for Primary Axillary Hyperhidrosis

Peangchan Balachandra[1] and Paisal Rummaneethorn [2*]

[12] *Department of Dermatology, Master of Science Program, School of Anti-aging and regenerative medicine, Mae Fah Luang University, Bangkok, 10240, Thailand*
[2]*Corresponding author, Dean of school of Anti-aging and regenerative medicine, Mae Fah Luang University, Bangkok, 10240, Thailand*
**Correspondind author. Tel.:+6626644361, E-mail: paisalrr@yahoo.com*

Abstract

Primary axillary hyperhidrosis (PAH) disturbs patients's daily life activity of patients and currently does not have gold standard for treatment yet. Intradermal botulinumtoxin typeA (BTXA) injection is effective, safe and have US-FDA approved for PAH treatment. Nevertheless this treatment is not permanent and possible side effects are bruising, and pain due to multiple injections. Currently patients' demand for non-invasive, no-downtime aesthetic and medical procedures is growing steadily. Radiofrequency (RF) is a noninvasive modality which causes skin hyperthermia. This might be an attractive modality for PAH treatment. A randomized, single-blinded , comparative study was conducted at Mah Fah Luang university hospital, Bangkok from January to April 2016. 20 men and women, aged 20 – 55 years old with diagnosis of PAH or have positive iodine starch test were enrolled and randomly assigned to the 40.68MHz noninvasive RF once a week for 4 sessions on one axilla and 50 units of intradermal BTXA injection at contralateral axilla at the first session. The primary outcome is assessed by iodine starch test photographic score. The secondary outcomes are Hyperhidrosis disease severity score (HDSS), patients' satisfactory score and side effects evaluation. Among the 20 enrolled participants, 17 subjects completed the study. The subjects were female 14/17 (82.35%) and male 3/17 (17.65%). The average participant age was 34.12 $\pm$5.12 years old. The result revealed that iodine starch test photographic score and HDSS of intradermal BTXA injection was significantly superior to noninvasive RF at 8th week and 12th week (p = 0.011 and < 0.001, respectively). HDSS of intradermal BTXA injection was also significantly superior to noninvasive RF at 8th week and 12th week. (p = 0.041 and < 0.002, respectively). Patient satisfactory scores of intradermal BTXA injection and noninvasive RF showed no significant difference. (p = 0.083). No severe adverse events were reported during the study. Intradermal BTXA injection has more efficacy for PAH treatment than noninvasive RF. Both treatments have no reported difference in patients satisfactory score. Both modalities have good safety profile.

Keywords: Noninvasive radiofrequency/ intradermal botulinumtoxin typeA injection/ primary axillary hyperhidrosis

Introduction

Axillary sweating contribute to controlling body temperature. However excessive sweating or hyperhidrosis can diminish the psychosocial, professional, and physical well-being of the patients. Axillary hyperhidrosis is a disorder of excessive sweating by the eccrine sweat glands, due to overactive cholinergic innervations.

Intradermal injection of botulinumtoxin typeA (BTXA) is effective, safe and have US-FDA approved for treating axillary hyperhidrosis. BTXA blocks acetylcholine release from cholinergic nerve fibers, which reduces excessive sweating. However, the treatment is not permanent. Possible side effects are bruising, and pain due to multiple injections (Naumann and Lowe , 2001)

Currently the demand of patients for non-invasive, no-downtime aesthetic and medical procedures is growing steadily. Radiofrequency (RF) is a non-invasive modality that causes skin hyperthermia. This may be an attractive modality for sweat gland thermolysis for PAH treatment.

Noninvasive RF destroy eccrine sweat glands by thermolysis. The rapidly oscillating electromagnetic RF field (40.68 MHz) emitted causes rapid rotation of the dielectric water molecules in the saturated hyperactive sweat glands resulting in frictional heat and sweat glands thermolysis. This mechanism leads to improvement in the degree of underarm sweating (Pinson and Olisova, 2013).

To date, no study has compare the efficacy and safety of noninvasive RF and intradermal BTXA

injection for treatment of PAH as well as the satisfaction of patient with those treatment modalities. Thus, the present study aims to compare the efficacy and safety of noninvasive RF and intradermal BTXA injection for treatment of PAH and also the satisfaction of patient with these two modalities

Objective

To evaluate the efficacy and safety of noninvasive RF compared to intradermal BTXA injection for treatment of PAH and also to compare the satisfaction of patients with these two modalities.

Research methodology

A randomized, single-blinded , comparative study was conducted at Mah Fah Luang university hospital, Bangkok from January to April 2016. 20 patients were enrolled. Inclusion criteria were (1) adult aged 20-55 years old and (2) a diagnosis of PAH or have positive iodine starch test . The study protocol was approved by local ethic committee of Mae Fah Luang university. Inform consent was obtained from all participants. The eligible participants were randomly assigned by a computer-generated randomization scheme, to undergo 40.68MHz noninvasive RF one week intervals for 4 sessions on one axilla and intradermal BTXA injection at contralateral axilla for 50 units once at the first session. Follow up appointments were 4, 8, and 12weeks after first session of treatment. The primary outcome was assessed by iodine starch test photographic score. The secondary outcomes were Hyperhidrosis disease severity score (HDSS), patients' satisfactory score and side effects evaluation.

Statistical Analysis

Demographic data of participants was present using appropriate descriptive statistics. Comparison for significant differences in iodine starch test photographic score, HDSS and patients' satisfactory score at 12th week between intradermal BTXA and noninvasive RF were sought by Wilcoxon Signed Rank Test. The side effects of both treatments were presented as appropriate descriptive statistics and significant differences were sought by McNemar Test. *p*-value < 0.05 was considered significant.

Results

Among the 20 enrolled participants, 17 subjects completed the study. The subjects were female 14/17 (82.35%) and male 3/17 (17.65%) . The average participant age was 34.12 ± 5.12 years.

The result revealed that iodine starch test photographic score of intradermal BTXA injection was significantly superior to noninvasive RF at 8th week and 12th week ($p = 0.011$ and < 0.001, respectively)(Figure 1) . and HDSS of intradermal BTXA injection was also significantly superior to noninvasive RF at 8th week and 12th week ($p = 0.041$ and < 0.002, respectively) (Figure 2) .

No significant difference in participant satisfaction was found between noninvasive RF and intradermal BTXA injection ($p = 0.083$) (Figure3) . No severe adverse events were reported or observed in any participants during the 12weeks of study.

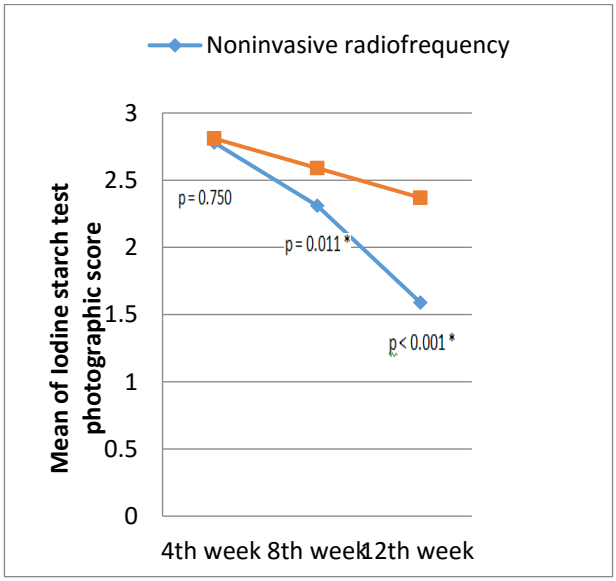


Figure1 Comparison mean of iodine starch test photographic score between noninvasive radiofrequency and intradermal botulinumtxin typeA injection at each week follow up

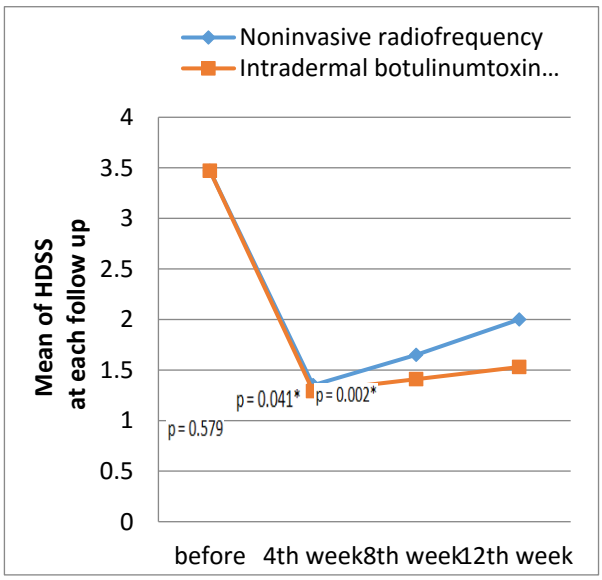


Figure2 Comparison mean of hyperhidrosis disease severity score of noninvasive radiofrequency and intradermal botulinum toxin typeA injection before and after treatment

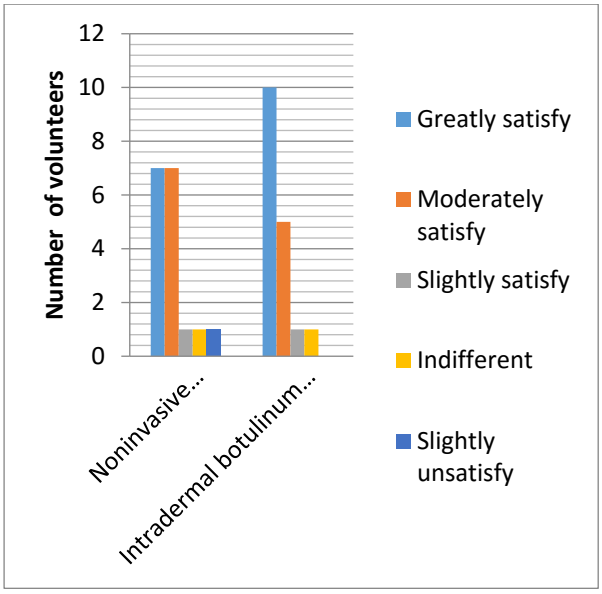
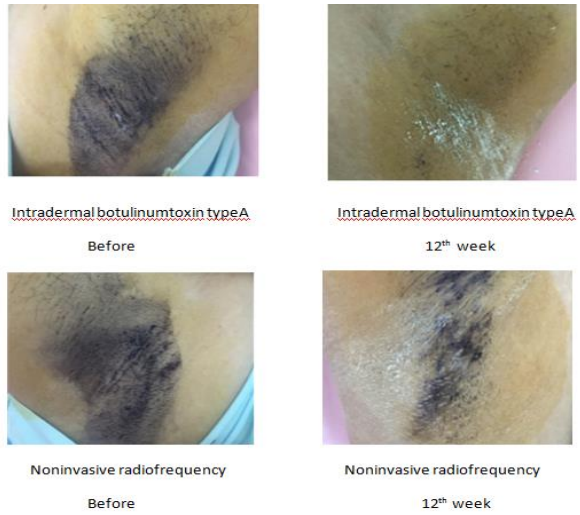


Figure3 Volunteers' satisfactory score in noninvasive radiofrequency and intradermal botulinumtoxin typeA at 12th week



Picture of iodine starch test before and after treatment with noninvasive radiofrequency and intradermal botulinumtoxin typeA at 12th week

Discussion

Intradermal BTXA 50 units/ axilla have efficacy and safety in PAH treatment by block acetylcholine release from cholinergic nerve fiber accordind to the study of Naumann and Lowe ,(2001). Currently patients' demand for non-invasive, no-downtime aesthetic and medical procedures is growing steadily. The study of Pinson and Olisova (2013) found that noninvasive RF has efficacy and safety on PAH too, But this modality never undergone statistical evaluation to compare the efficacy and safety in PAH with the standard treatment which is intradermal BTXA injection yet, So this study conduct to compare efficacy and safety between intradermal BTXA and noninvasive RF. And the result were

Consider at each follow up appointment found that

At 4th week follow up, intradermal BTXA injection did not have statistical significant in decreasing degree of hyperhidrosis with noninvasive RF in iodine starch test photographic score (p-value = 0.750), and HDSS (p = 0.579).

At 8th week follow up, intradermal BTXA injection had statistical significant in decreasing degree of hyperhidrosis superior to noninvasive RF in iodine starch test photographic score (p = 0.011), and HDSS (p = 0.041).

At 12th week follow up, intradermal BTXA injection had statistical significant in decreasing degree of hyperhidrosis superior to noninvasive RF in iodine starch test photographic score (p < 0.001), and HDSS (p = 0.002).

Consider volunteers satisfactory score after 12 weeks after treatment found that Patients' satisfactory of intradermal BTXA injection and noninvasive RF are not different (p = 0.083).

Volunteers who underwent noninvasive RF have only mild heating sensation 6 volunteers,equal to 35.29 percent. 2 volunteers have mild redness and mild erythema equal to 11.76 percent. 1 volunteer has mild pain equal to 5.88 percent. Unlike previous study of Pinson and Olisova (2013) found that volunteers had heating sensation 55.32 percent , redness and erythema equal to 26.76 percent. Researcher assume that percent of heating sensation in this study was lower than previous study because in this study volunteers received topical anesthesia before treatment while previous treatment didn't received any anesthesia.

Volunteers who underwent intradermal BTXA injection group have mild pain 5 persons equal to 29.11 percent, 1 volunteer mild bruising, equal to 5.88 percent while study of Naumann and Lowe (2001) found that volunteers had mild pain 35.11 percent, common cold 2.35 percent, compensatory sweating 1.39 percent, and no bruising was reported.

Conclusion

Intradermal BTXA injection could accomplish significantly better outcome than noninvasive RF in iodine starch test photographic score and HDSS at 8th week and 12th week.

And paients' satisfactory of score at 12th week of intradermal BTXA injection and noninvasive RF are not different. No recorded severe side effects.

Such provided information suggest that intradermal BTXA injection have efficacy and for PAH superior to noninvasive RF. Both intradermal BTXA

and noninvasive RF have high safety profile for PAH treatment.

References

Strutton DR, Kowalski JW, Glaser DA et al. US prevalence of hyperhidrosis and impact on individuals with axillary hyperhidrosis: results from a national survey. J Am Acad Dermatol 2004;51:241–248

Gelbard CM, Epstein H, Hebert A. Primary pediatric hyperhidrosis: a review of current treatment options.Pediatr Dermatol 2008;25:591–598

Heckmann M, Ceballos-Baumann AO, Plewig G. Botulinum toxin A for axillary hyperhidrosis (excessive sweating). N Engl J Med 2001;344:488–493

Naumann M, Lowe NJ. Botulinum toxin type A in treatment of bilateral primary axillary hyperhidrosis: randomised, parallel group, double blind, placebo controlled trial. BMJ 2001;323:596–599

Shelley, W.B.; Hurley, H.J., Jr. Studies on topical antiperspirant control of axillary hyperhidrosis. *Acta Derm. Venereol.* **1975**, *55*, 241–260

Bushara, K.O.; Park, D.M.; Jones, J.C.; Schutta, H.S. Botulinum toxin—A possible new treatment for axillary hyperhidrosis. *Clin. Exp. Dermatol.* **1996**, *21*, 276–278

Jankovic, J. Treatment of cervical dystonia with botulinum toxin. *Move. Disord.* **2004**, *19*,109–115

Atkins JL, Butler PE. Hyperhidrosis: a review of current management. Plast Reconstr Surg. 2002;110:222-228

Emilia del Pino M, Rosado RH, Azuela A, Graciela Guzman M. Effect of controlled volumetric tissue heating with radiofrequency on cellulite and the subcutaneous tissue of the buttocks and thighs. J Drugs Dermatol. 2006;5:714-722

Comparative Choleesteroal-Lowering Effects of Combination of Oat Beta-Glucan and Policosanol Versus Oat Beta-Glucan alone in A population of Hypercholesterolemin Men and Women

Chanida Bhanubandh Na Ayudhya[1], Karnt Wongsuphasawat[1]*, Thamthiwat Nararatwanchai[1], Somboon Roongphornchai[1], Rassapoom Sumaetheiwit[2], Atchima Suwanchinda[2], Tanomkit Pawcsuntorn[2], Phaisal Rummaneetorn[2]

[1]*Department of Anti-Aging and Regenerative Medicine, School of Anti-Aging and Regenerative Medicine, Mae Fah Luang University, Bangkok, Thailand*
[2]*Department of Dermatology, School of Anti-Aging and Regenerative Medicine, Mae Fah Luang University, Bangkok, Thailand*
**Corresponding author, Email: karnt.wong@mfu.ac.th*

ABSTRACT

This study aimed to compare the cholesterol-lowering effects of the combination of Oat Beta-Glucan and Policosanol versus Oat Beta-Glucan alone in a population of hypercholesterolemia men and women. 60 participants with LDL-C 130-190 mg/dL had divided into two groups by randomization. The experimental group had been received Oat Beta-glucan 3 g/d along with Policosanol 20 mg/d as they split into two meals a day for 12 weeks. The control group had been received Oat Beta-glucan 3 g/d along with Placebo as they splitted into two meals a day for 12 weeks. Both groups had tendency of LDL-C level to be decreased from all four periods of times. When comparing to LDL-C level within the same group, it was found that in the experimental group, the LDL-C had decreased significantly since the 4th week onwards as $p=0.001$, $p<0.001$, and $p<0.001$, respectively. The controlled group had decreased significantly since the 4th week onwards with $p<0.001$. On the other hand, when comparing to each periods between two groups, there were no statistically difference in all four periods. No significant reduction showed in the 12th week between both groups; the experimental group was -29.12 mg/dl (19.59%) versus the controlled group was -24.88 mg/dl (16.31%) ($p=0.43$). Consequently, the combination of the Oat Beta-Glucan and Policosanol was no more significant effective in lowering cholesterol than the Oat Beta-Glucan alone over 12 weeks in a population of hypercholesterolemia which is required further clinical research studies in longer intervention periods.

Keywords: *Hypercholesterolemia; Oat Beta Glucan; Policosanol*

Introduction

Coronary heart disease (CHD) is a leading cause of ailment and fatality globally. Hypercholesterolemia, one of the major risk factors can be identified by elevated levels of low-density lipoprotein (LDL). Despite preventive strategies, increase of aging populations and progressively change of inactive lifestyles, CHD is likely to increase globally. Since hypercholesterolemia is a major risk factor, it is much important to manage effectively (Tabesh *et al.,* 2014; Mannu *et al.,* 2012). Many studies have found the potential of diets and dietary elements as first-line interference in the prevention and treatment of hypercholesterolemia. Evidence shows that more intakes of dietary soluble fibres such as beta-glucan can effectively lower the risk of CVD through the risk factors such as hypercholesterolemia, which significantly lower LDL-cholesterol (LDL-C). It has been reported that 1% reduction in serum LDL level is associated with 1% to 2% decrease of CVD occurrence (Tabesh *et al.,* 2014).

Another interesting component is Policosanol, a mixture of higher primary aliphatic alcohols isolated from sugar cane wax, whose main component is octacosanol. Policosanol has been shown to lower cholesterol in animal models, healthy volunteers, and patients with type II hypercholesterolemia (Gouni-Berthold and Berthold, 2001). Therefore, the purpose of this study was to combine oat beta-glucan with other dietary means of controlling blood sugar, and to consequently prevent the need for cholesterol-lowering drugs in hyperlipidemic patients (Khoury *et al.,* 2012). This study aimed to compare the cholesterol-lowering effects of the combination of Oat Beta-Glucan and Policosanol versus Oat Beta-Glucan alone in a population of hypercholesterolemia men and women.

Materials and Methods

Population

This study included 60 subjects (males and females) aged 25-50 years old, who had LDL-cholesterol 130-190 mg/dL and agreed to join the study.

Sample size

Sample size was defined from the study of the consumption of bread enriched with beta glucan reduces LDL-cholesterol and improves insulin resistance in patients with type 2 diabetes (Liatis *et al.*, 2009)

$$n = \frac{(Z_a + Z_b)^2.(s_1^2 + s_2^2)}{(m_1 - m_2)^2}$$

Confident interval was set at 95% α = 0.05

$$n = \frac{(1.645+1.282)^2(0.80^2 + 0.45^2)}{((-0.66)-(-0.11))^2}$$

$$n = 24$$

As the allowance for 25% drop out rate, the sample size was set 30 subjects per group. Hence, the total sample size was 60 subjects.

Research Design

This study was the clinical experiment using the randomized double blind controlled trial. Independent variables were the given treatment including Oat beta-glucan alone and Oat beta-glucan plus Policosanol. Dependent variables were total cholesterol and LDL-cholesterol. Subjects were pregnancy and lactating women.

Materials

Materials included (a) Oat Beta-Glucan 3 g/d plus Policosanol 20 mg/d, (b) Oat Beta-Glucan 3 g/d plus Placebo capsule. The dosage of OBG and Policosanol used based on previous experience using in human study of the health benefits (Liatis *et al.*, 2009; Gouni-Berthold and Berthold, 2001), (c) Blood collecting test (TC, LDL-C, HDL-C, TG), (d) Research protocol declaration, (e) Informed consent form, (f) Investigator record form (Physical and laboratory examination), and (g) Subject record form (History questionnaire, Daily diet).

Research Procedures

Subjects were recruited according to the inclusion and exclusion criteria. All subjects were given all information about research information, dietary instruction and follow up schedule throughout the study. All subjects signed of the inform consent. All subjects had undergone medical history record and physical examination including blood pressure, weight, height and body mass index (BMI). Blood sample were taken for the laboratory tests including TC, LDL-C, HDL-C and TG, respectively. All subjects were divided into two groups by randomization; the control group had Oat Beta-Glucan 3 g/d plus Policosanol 20 mg/d and the experimental group had Oat Beta-Glucan 3 g/d plus Placebo capsule. The duration of this study lasted 12 weeks. All subjects had to return to the clinic for evaluation of any side-effects, compliance, blood pressure, weight, height, BMI, TC, LDL-C, HDL-C and TG every the 4th , 8th and 12th week. Blood sample were also obtained using standard venepuncture techniques after fasting for 12 hours.

Statistical Analysis

This study used the Readymade Data Analysis Program for data analysis as follows. Describing Statistic: was used to describe the general characteristics of samples. Qualifying data included gender, overweight-fat condition, statistically report, quantity and percentage. In addition, the quantifying statistic included age, body weight condition, Total Cholesterol, LDL Cholesterol, HDL Cholesterol, Triglyceride, Systolic Blood Pressure, Diastolic Blood Pressure, AST and ALT levels. Report with percentage and standard distracting was also employed. Deducing Statistic included (a) comparing the general characteristics between the experimental group and the control group by using qualifying data with statistic such as Chi-square/Fisher's exact test. The quantifying data at the point of beginning used student t-test for data analysis, (b) comparing the body weight, Total Cholesterol, LDL Cholesterol, HDL Cholesterol, Triglyceride, Systolic Blood Pressure, Diastolic Blood Pressure which had been measured repeatedly in four periods. AST and ALT Levels was repeatedly measured in two times between the experimental group and control group with statistic such as the Repeated ANOVA measurements. Finally, every experiments determined the significantly and statistically at $p<0.05$.

Results

General Characteristics of Samples

According to table 1, it was found that most of subjects were female, 72% of experimental group and 76.9% of control group. Averaged ages of two groups were similar in which 35.32±8.08 years old of experimental group and 38.69±7.96 years old of control group. For those subjects who were overweight or fat (BMI ≥ 25.0 kg/m^2), the experimental group was found 20% while the control group was found 15.4% in which the above general characteristic was not statistically different similar to body weight information, Total Cholesterol Level, LDL Cholesterol, HDL Cholesterol, Triglyceride, Systolic Blood Pressure, Diastolic Blood Pressure, AST and ALT, respectively.

Table 1 General characteristic of the control group (Oat Beta-glucan plus Placebo) and the experimental group (Oat Beta-glucan plus Policosanol)

General Characteristics	Oat Beta-glucan plus Policosanol (n=25)		Oat Beta-glucan plus Placebo (n=26)		p-value
	n	%	n	%	
Gender					0.69
Male	7	28.0	6	23.1	
Female	18	72.0	20	76.9	
Age (Year)	35.32±8.08		38.69±7.96		
Body Overweight (BMI ≥25.0 kg/m2)	5	20.0	4	15.4	0.73
Body Weight	60.20±9.20		59.62±9.58		0.33
Total cholesterol (mg/dl)	230.72±27.70		228.85±17.13		0.57
LDL cholesterol (mg/dl)	147.64±19.06		148.31±14.79		0.83
HDL cholesterol (mg/dl)	60.56±9.15		60.92±8.41		0.89
Triglyceride (mg/dl)	88.80±30.40		104.12±44.86		0.13
Systolic blood pressure (mmHg)	117.00±8.85		121.19±8.33		0.13
Diastolic blood pressure (mmHg)	74.44±5.39		76.69±6.79		0.61
Aspartate aminotransferase (AST)	23.96±8.01		22.50±5.05		0.44
Alanine aminotransferase (ALT)	21.44±8.73		24.19±8.42		0.26

LDL Cholesterol Level

Table 2 Comparison of LDL cholesterol level between the control group (Oat Beta-glucan plus Placebo) and the experimental group (Oat Beta-glucan plus Policosanol)

Periods	Oat Beta-glucan plus Policosanol (n=25)		Oat Beta-glucan plus Placebo (n=26)		p-value between group
	Mean	SD	Mean	SD	
Beginning	147.64	19.06	148.31	14.79	0.89
4th week	136.40	25.85	136.23	19.39	0.98
8th week	127.08	24.18	130.50	20.88	0.59
12th week	118.52	24.21	123.42	22.26	0.45
p-value within group					
Beginning vs. 4th week	0.001***		<0.001***		
Beginning vs. 8th week	<0.001***		<0.001***		
Beginning vs. 12th week	<0.001***		<0.001***		
Difference:					
4thweek-Beginning	-11.24	12.08	-12.08	15.00	0.83
8thweek-Beginning	-20.56	20.84	-17.81	21.44	0.64
12thweek-Beginning	-29.12	21.42	-24.88	24.81	0.52
Percentage of changes in 12th week	-19.59	13.99	-16.31	15.66	0.43

According to table 2, it was found that both the experimental group and the control group had tendency of LDL Cholesterol level to be decreased from all four periods of measurements. On the other hand, when comparing to LDL cholesterol level in each period of time between these two groups, there was no statistically difference in all four period of measurements (p=0.89, 0.98, 0.59, and 0.45, respectively). When comparing to LDL cholesterol level within the same group, it was found that in the experimental group, LDL cholesterol had decreased significantly since the 4th week onwards (p=0.001, <0.001, and <0.001, respectively) when comparing from the beginning point. Similarly to the control group which was found that LDL cholesterol level had decreased significantly since the 4th week onwards with p<0.001. When comparing between the 4th, 8th and 12th week, both groups had an average of LDL cholesterol level which was not statistically different as p=0.83, 0.64, and 0.52, respectively.

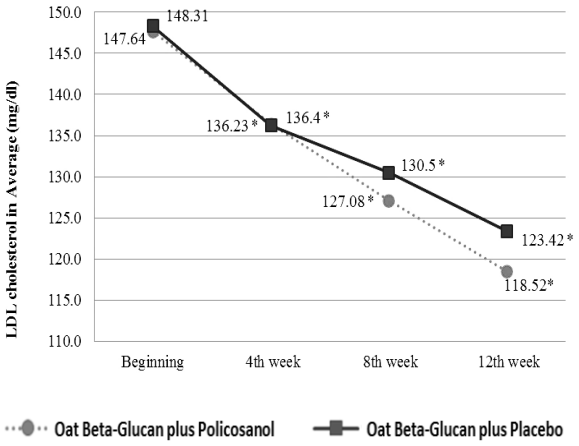


Figure 1 Comparison of LDL cholesterol level between the control group (Oat Beta-glucan plus Placebo) and the experimental group (Oat Beta-glucan plus Policosanol) at the beginning, 4th week, 8th week, and 12th week, respectively.

Total Cholesterol Level

Table 3 Comparison of total cholesterol level between the control group (Oat Beta-glucan plus placebo) and the experimental group (Oat Beta-glucan plus Policosanol)

Periods	Oat Beta-glucan plus Policosanol (n=25)		Oat Beta-glucan plus Placebo (n=26)		p-value between group
	Mean	**SD**	**Mean**	**SD**	
Beginning	230.72	27.70	228.85	17.13	0.77
4th week	222.16	32.84	217.35	22.31	0.54
8th week	208.64	29.57	209.19	21.11	0.94
12th week	198.04	31.21	199.85	21.87	0.81
p-value within group					
Beginning vs. 4th week	0.13		0.01*		
Beginning vs. 8th week	<0.001***		<0.001***		
Beginning vs. 12th week	<0.001***		<0.001***		
Difference:					
4thweek-Beginning	-8.56	19.56	-11.50	16.70	0.57
8thweek-Beginning	-22.08	22.56	-19.65	21.62	0.70
12thweek-Beginning	-32.68	28.61	-29.00	23.36	0.62
Percentage of changes in 12th week	-13.87	11.60	-12.42	9.97	0.63

According to table 3, it was found that the total cholesterol level was decreased in all four periods of measurements in both groups. Although, when comparing the average of total cholesterol level in each period of time between two groups, it did not find any statistically difference in all four periods (p=0.77, 0.54, 0.94, and 0.81, respectively). When comparing the total cholesterol level within the same group, it was found that the total cholesterol was decreased in the experimental group furtively and statistically since in the 8th week onward with p<0.001 similar to the control group in which the total cholesterol level was decreased furtively and statistically since in the 4th week onward with p=0.01, <0.001, and <0.001, respectively. On the other hand, it was found that the changes of total cholesterol level in the 4th, 8th and 12th week in both groups was not statistically difference with p=0.57, 0.70, and 0.62, respectively.

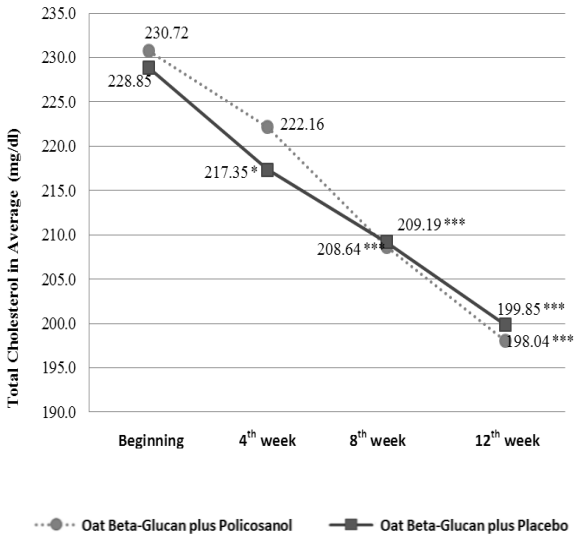


Figure 2 Comparison of the total cholesterol level between the control group (Oat Beta-glucan plus Placebo) and the experimental group (Oat Beta-glucan with Policosanol) at the beginning, 4th week, 8th week, and 12th week, respectively.

The Body Weight

According to table 4, it was found that the experimental group tended to lose body weights the 8th week and 12th week while the control group had quite stable body weight in all periods of times. When comparing the averaged body weight in each period of time between two groups, it was found that there was no statistically difference from all four periods of times (p=0.83, 0.86, 0.84, and 0.82, respectively). When comparing the body weight in the same group in different timing, it was found that both the experimental group and the control group had a stable body weight, but did not find any statistically difference from the beginning through the 12th week. It also found that the change of the body weight from the beginning to the 4th, 8th, and 12th week had no statistically difference at p=0.33, 0.87 and, 0.99, respectively.

Table 4 Comparison of the body weight between the control group (Oat Beta-glucan plus Placebo) and the experimental group (Oat Beta-glucan plus Policosanol)

Periods	Oat Beta-glucan plus Policosanol (n=25)		Oat Beta-glucan plus Placebo (n=26)		p-value between group
	Mean	SD	Mean	SD	
Beginning	60.20	9.20	59.62	9.58	0.83
4th week	60.22	9.33	59.41	9.53	0.76
8th week	59.86	9.23	59.32	9.47	0.84
12th week	59.84	8.88	59.25	9.39	0.82
p-value within group					
Beginning vs. 4th week	1.000		1.000		
Beginning vs. 8th week	0.606		0.895		
Beginning vs. 12th week	0.743		0.669		
Difference: 4thweek-Beginning	0.04	0.70	-0.19	0.97	0.33
8thweek-Beginning	-0.34	1.08	-0.29	0.95	0.87
12thweek-Beginning	-0.36	1.28	-0.37	1.01	0.99
Percentage of changes at 12th week	-0.54	2.03	-0.56	1.57	0.98

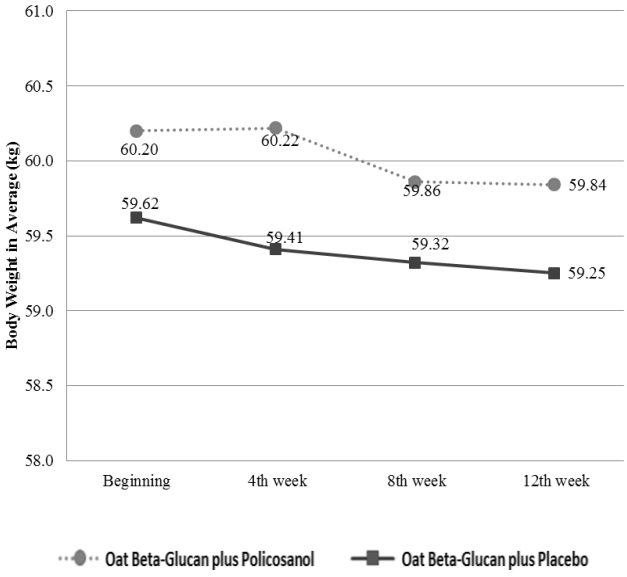


Figure 3 Comparison of the body weight between the control group (Oat Beta-glucan plus Placebo) and the experimental group (Oat Beta-glucan with Policosanol) at the beginning, 4th week, 8th week, and 12th week, respectively.

HDL Cholesterol Level

Table 5 Comparison of HDL cholesterol between the control group (Oat Beta-glucan plus Placebo) and the experimental group (Oat Beta-glucan plus Policosanol)

Periods	Oat Beta-glucan plus Policosanol (n=25)		Oat Beta-glucan plus Placebo (n=26)		p-value between group
	Mean	SD	Mean	SD	
Beginning	60.56	9.15	60.92	8.41	0.88
4th week	60.52	10.30	60.54	7.15	0.99
8th week	58.92	8.90	59.19	5.81	0.90
12th week	56.88	10.40	57.85	8.17	0.71
p-value within group					
Beginning vs. 4th week	1.00		1.00		
Beginning vs. 8th week	1.00		1.00		
Beginning vs. 12th week	0.47		0.79		
Difference: 4thweek-Beginning	-0.04	10.84	-0.38	6.89	0.89
8thweek-Beginning	-1.64	10.93	-1.73	7.51	0.97
12thweek-Beginning	-3.68	9.13	-3.08	11.22	0.83
Average of changes in 12th week	-5.33	14.56	-2.92	21.17	0.64

According to table 5, it was found that both the experimental group and the control group had tendency of HDL cholesterol level to be steady from all four periods of times. On the other hand, when comparing HDL cholesterol level in average of each period between two groups, it found no statistically differences as p=0.88, 0.99, 0.90, and 0.71, respectively. When comparing the HDL cholesterol levelwithin the same group, it was found that the experimental group had no statistically difference as p=1.00, 1.00, and 0.47, respectively, in the 4th, 8th and 12th week when compared to the beginning. Similar to the control group which found that HDL cholesterol level had not changed statistically from all three periods of times as p=1.00, 1.00, and 0.79, respectively. While the changes of HDL cholesterol level in the 4th, 8th and 12th week of both groups had not statistically different as p=0.89, 0.97, and 0.83, respectively.

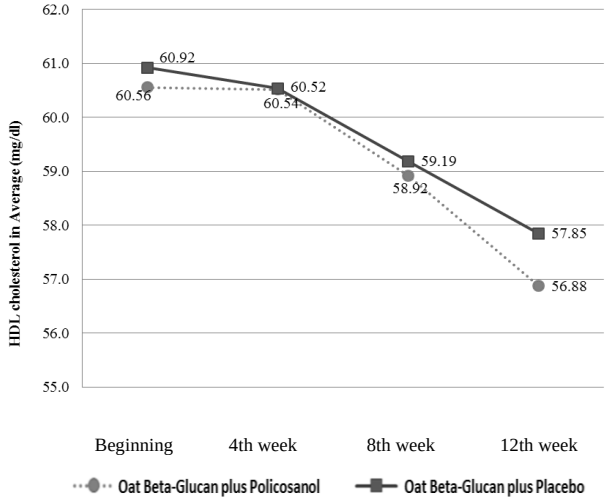


Figure 4 Comparison of HDL cholesterol level between the control group (Oat Beta-glucan plus Placebo) and the experimental group (Oat Beta-glucan with Policosanol) at the beginning, 4th week, 8th week, and 12th week, respectively.

Triglyceride Level

Table 6 Comparison of Triglyceride level between the control group (Oat Beta-glucan plus Placebo) and the experimental group (Oat Beta-glucan plus Policosanol)

Periods	Oat Beta-glucan plus Policosanol (n=25)		Oat Beta-glucan plus Placebo (n=26)		p-value between group
	Mean	SD	Mean	SD	
Beginning	88.80	30.40	104.12	44.86	0.16
4th week	92.48	28.49	97.85	32.51	0.53
8th week	89.68	31.71	95.08	30.67	0.54
12th week	101.92	60.28	89.15	32.02	0.35
p-value within group					
Beginning vs. 4th week	1.00		1.00		
Beginning vs. 8th week	1.00		1.00		
Beginning vs. 12th week	1.00		1.00		
Difference:					
4thweek–Beginning	3.68	16.95	-6.27	27.65	0.13
8th week–Beginning	0.88	26.31	-9.04	39.22	0.30
12thweek–Beginning	13.12	50.46	-14.96	46.87	0.04
Percentage of 12th week	14.49	48.42	-5.38	35.87	0.10

According to table 6, it was found that both the experimental group and the control group had steady tendency of triglyceride level in all four periods of times. On the other hand, when comparing to the average of each period between two group, it found no statistically difference p=0.16, 0.53, 0.54, and 0.35, respectively. When comparing the triglyceride level within the same group, it was found that the triglyceride level in the experimental group had no statistically changes from all three periods of time as p = 1.00 from the beginning. Similar to the control group which found no statistically changes from all three periods of times as p=1.00, 1.00, and 0.74, respectively. The changes of the triglyceride level in the 4th and 8th week of both groups had no statistically difference at p=0.13 and 0.30, respectively. On the other hand, it found statistically differences between both groups in the 12th week as p=0.04, in which the experimental group increased (13.12±50.64) while the control group decreased (14.96±46.87) from the beginning of time.

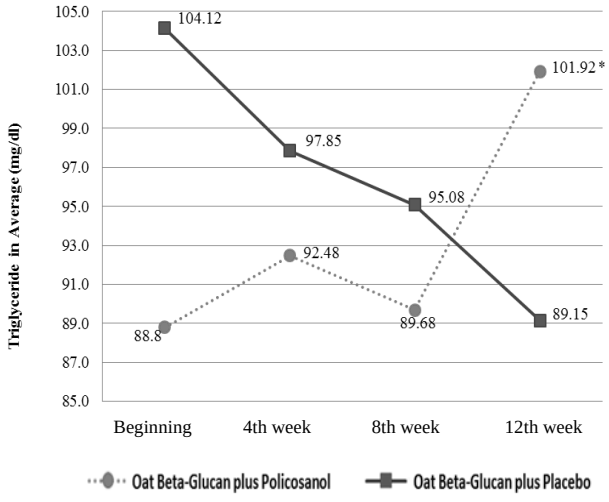


Figure 5 Comparison of the triglyceride level between the control group (Oat Beta-glucan plus Placebo) and the experimental group (Oat Beta-glucan with Policosanol) at the beginning, 4th week, 8th week, and 12th week, respectively.

Systolic Blood Pressure Level

Table 7 Comparison of systolic blood pressure level between the control group (Oat Beta-glucan plus placebo) and the experimental group (Oat Beta-glucan plus Policosanol)

Periods	Oat Beta-glucan plus Policosanol (n=25)		Oat Beta-glucan plus Placebo (n=26)		p-value between group
	Mean	SD	Mean	SD	
Beginning	117.00	8.85	121.19	8.33	0.09
4th week	119.84	9.76	122.69	6.37	0.22
8th week	119.04	9.26	120.69	7.17	0.48
12th week	118.60	9.62	121.85	8.03	0.20
p-value within group					
Beginning vs. 4th week	0.26		1.00		
Beginning vs. 8th week	0.28		1.00		
Beginning vs. 12th week	0.90		1.00		

According to table 7, it was found that the experimental group and the control group had a steady tendency of systolic blood pressure for all periods of times. When comparing the average of systolic blood pressure from each period between two groups found no statistically difference from all four periods as p=0.09, 0.22, 0.48, and 0.20, respectively. When comparing to systolic blood pressure level within the same group found that the experimental group towards systolic blood pressure level didn't have any statistically changes for all three periods as p=0.26, 0.28, and 0.90, respectively, when comparing from the beginning point. While the control group showed systolic blood pressure level steady at p=1.00 when comparing to the beginning point from all three periods.

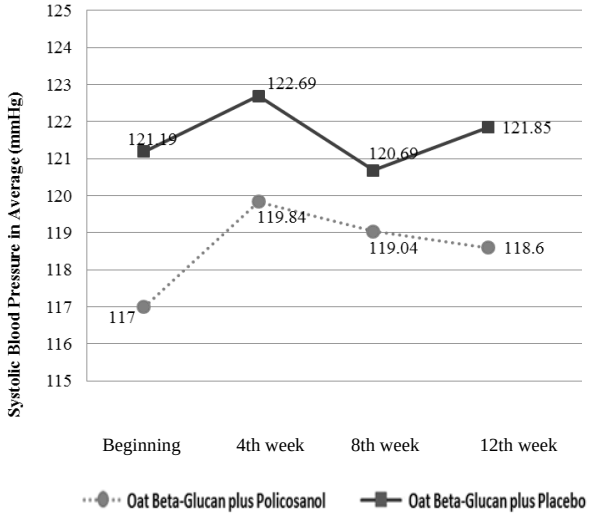


Figure 6 Comparison of the systolic blood pressure level between the control group (Oat Beta-glucan plus Placebo) and the experimental group (Oat Beta-glucan with Policosanol) at the beginning, 4th week, 8th week, and 12th week, respectively.

Diastolic Blood Pressure Level

Table 8 Comparison of diastolic blood pressure level between the control group (Oat Beta-glucan plus placebo) and the experimental group (Oat Beta-glucan plus Policosanol)

Periods	Oat Beta-glucan plus Policosanol (n=25)		Oat Beta-glucan plus Placebo (n=26)		p-value between group
	Mean	SD	Mean	SD	
Beginning	74.44	5.39	76.69	6.79	0.20
4th week	74.80	5.39	76.23	5.67	0.36
8th week	75.36	4.64	75.73	4.68	0.78
12th week	75.48	6.08	74.62	6.01	0.61
p-value within group					
Beginning vs. 4th week	1.00		1.00		
Beginning vs. 8th week	1.00		1.00		
Beginning vs. 12th week	1.00		0.76		

According to table 8, it was found that the experimental group and the control group had a steady tendency of diastolic blood pressure level for all periods of times. When comparing the average of diastolic blood pressure level in average from each period between two groups found no statistically difference from all four periods as p=0.20, 0.36, 0.78, and 0.61, respectively. When comparing to the diastolic blood pressure level within the same group found that the experimental group showed no statistically changes over three periods as p=1.00 when comparing to the beginning point. Similar to the control group that the diastolic blood pressure level steady as p=1.00, 1.00, 0.76, respectively, when comparing to the beginning point.

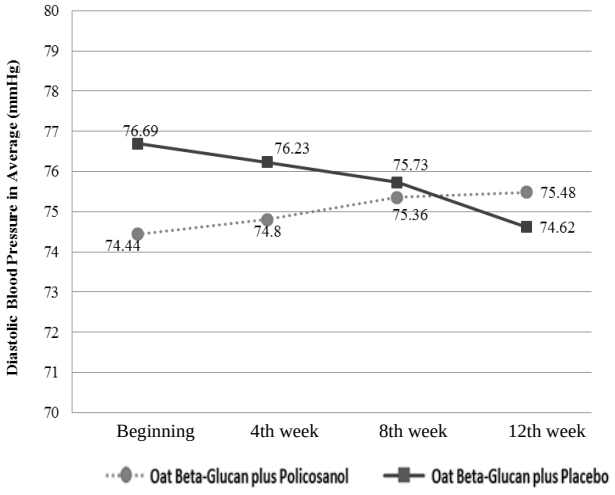


Figure 7 Comparison pf the Diastolic Blood Pressure Level between the control group (Oat Beta-glucan plus placebo) and the Experimental group (Oat Beta-glucan with Policosanol) at the beginning, 4th week, 8th week, and 12th week, respectively.

Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) Level

Table 9 Comparison of AST and ALT level between the control group (Oat Beta-glucan plus Placebo) and the experimental group (Oat Beta-glucan plus Policosanol)

Periods	Oat Beta-glucan plus Policosanol (n=25)		Oat Beta-glucan plus Placebo (n=26)		p-value between group
	Mean	SD	Mean	SD	
Aspartate aminotransferase (AST)					
Beginning	23.96	8.01	22.50	5.05	0.44
12th week	22.96	7.66	21.35	3.47	0.33
p-value within group	0.07		0.03*		
Difference:12th week - Beginning	-1.00	2.20	-1.15	3.09	0.84
Percentage of changes in 8th week	-3.65	9.26	-3.86	10.76	0.94
Alanine aminotransferase (ALT)					
Beginning	21.44	8.73	24.19	8.42	0.26
12th week	20.56	8.20	22.92	7.50	0.29
p-value within group	0.18		0.049*		
Difference:12th week - Beginning	-0.88	3.79	-1.27	2.60	0.67
Percentage of changes in 8th week	-1.11	22.03	-3.65	11.10	0.60

According to table 9, it was found that both experimental group and control group had a tendency of AST and ALT levels to be decreased from the beginning. On the other hand, when comparing AST and ALT levels in average of each period between two groups found no statistically difference. And when considering to the timing it found that timing had effect to AST and ALT levels causing the changes of ASL and ALT levels decreased. Thus, when comparing AST and ALT levels within the same group, it was found that in the experimental group, AST and ALT levels had decreased from the beginning but not significantly p=0.07 in AST and p=0.18 in ALT, respectively. While the control group, AST and ALT levels had decreased from the beginning statistically significantly at p=0.03 in AST and p=0.049 in ALT, respectively. The changes of AST and ALT levels showed that, in 12th week, both groups had no statistically difference at p=0.84 in AST and 0.67 in ALT, respectively.

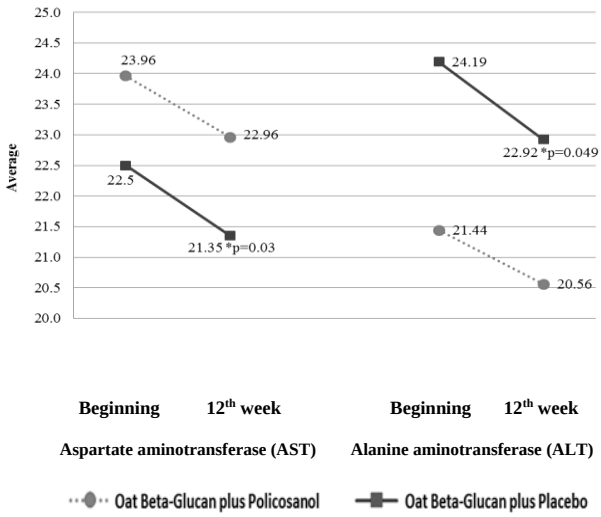


Figure 8 Comparison of AST and ALT Level between the control group (Oat Beta-glucan plus Placebo) and the experimental group (Oat Beta-glucan with Policosanol) at the beginning, 4th week, 8th week, and 12th week, respectively.

Discussion

According to the result of this study on LDL Cholesterol, both the experimental group and the control group had statistically decreased of LDL cholesterol level from all four periods of times. But the experimental group was no more significant effectively in lowering cholesterol than control group over 12 weeks. Moreover, the experimental group had a tendency for the cholesterol level to be decreased lower than the control group. Thus when the experiment is continuing in a longer period, a more obvious result might be found.

Comparing with the previous study, the consumption of bread enriched with beta-glucan reduces LDL-cholesterol and improves insulin resistance in patients with type 2 diabetes (Liatis *et al.*, 2009). This previous studies has shown that the water-soluble dietary fibre beta-glucan, a natural component of oats, reduces cholesterol and postprandial hyperglycaemia. The aim of the present study was to investigate the effect of beta-glucan-enriched bread consumption on the lipid profile and glucose homoeostasis of patients with type 2 diabetes (T2D). For the results, the consumption of bread containing beta-glucan led to significant reductions (vs the control group) in LDL-C of 0.66 mmol/l (15.79%) versus 0.11 mmol/l (2.71%) (P = 0.009), in total cholesterol of 0.80 mmol/l (12.80%) versus 0.12 mmol/l (1.88%) (P = 0.006). This study concluded that beta-glucan enriched bread may contribute to the improvement of the lipid profile and insulin resistance in patients with T2D. Another study of Meta-analysis of the effect of beta-glucan intake on blood cholesterol and glucose levels (Tiwari and Cummins, 2010). This study concluded that

consumption of 3 g/d of oat or barley b-glucan is sufficient to decrease blood cholesterol levels.

For the present study using Oat Beta-glucan 3 gram/day along with Policosanol 20 milligram/day for experimental group and Oat Beta-glucan 3 gram/day along with Placebo for control group, both groups were given the same dosage used in the previous studies (Gouni-Berthold and Berthold, 2001; Tiwari and Cummins, 2010; Liatis *et al.*, 2009). It was found that the consumption of oat beta-glucan plus policosanol led to significant reductions (vs the control group) in LDL-C of 19.59 % versus 16.31% (P = 0.43). In the experimental group the LDL cholesterol percentage level was more decreased than the control group and the previous study (Gouni-Berthold and Berthold, 2001; Tiwari and Cummins, 2010; Liatis *et al.*, 2009) using oat beta-glucan or policosanol solely. When comparing control group to the previous study, it was decreased slightly. On the other hand, it was found that the consumption of oat beta-glucan plus policosanol led to significant reductions (vs the control group) in total cholesterol of 13.87% versus 12.42% (P = 0.63). In the experimental group, the total cholesterol percentage level was more decreased than control group and previous study (Gouni-Berthold and Berthold, 2001; Tiwari and Cummins, 2010; Liatis *et al.*, 2009) using oat beta-glucan or policosanol solely. When comparing control group to the previous study, only slightly reductions were found in both groups. When analysed result the change of AST ALT level over 12 weeks period found that the experimental group had no significant decrease from the beginning, which mean that Oat Beta-Glucan and Policosanol are safe for not disturbing liver function. While the control trial had significant decrease from the beginning in AST and ALT, which mean that Oat Beta-Glucan solely is significant to decrease of AST ALT level over 12 weeks period, which seem to be good for liver function. According to the result of this study, both the experimental group and the control group had tendency of LDL cholesterol level to be decreased from all four period of measurements. Moreover, the experimental group had a tendency for the cholesterol level to be decreased lower than the control group. Thus when the experiment is continuing in a longer period, a more obvious result might be found.

Conclusion

This study aimed to compare the cholesterol-lowering effects of the combination of Oat Beta-Glucan and Policosanol versus Oat Beta-Glucan alone in a population of hypercholesterolemia men and women. The combination of the Oat Beta-Glucan and Policosanol was no more significant effective in lowering cholesterol than the Oat Beta-Glucan alone over 12 weeks in a population of hypercholesterolemia which is required further clinical research studies in longer intervention periods.

References

Gouni-Berthold, I. and Berthold, H.K. (2001). Policosanol: Clinical Pharmacology and therapeutic significance of a new lipid-lowering agent. American Heart Journal, 143(2), 356-365.

Khoury, D.E., Cuda, C., Luhovyy, B.L., and Anderson G.H. (2012). Beta Glucan: Health Benefits in Obesity and Metabolic Syndrome

Liatis, S., Tsapogas, P., Chala, E., Dimosthenopoulos, C., Kyriakopoulos, K., Kapantais K. and Katsilambros, N. (2009). The consumption of bread enriched with beta glucan reduces LDL-cholesterol and improves insulin resistance in patients with type 2 diabetes. Diabetes and Metabolism, 35, 115–120.

Mannu, G.S., Zaman, M.J., Gupta, A, Rehman, H.U. and Myint, P.K. (2012). Expert Review Cardiovascular Therapy, 10(10), 1239-1249.

Tabesh, Faezeh Sanei, Hamid Jahangiri, Mansour Momenizadeh, Amir Tabesh, Elham Pourmohammadi, Kiana Sadeghi, Masoumeh (2014). The effects of beta-glucan rich oat bread on serum nitric oxide and vascular endothelial function in patients with hypercholesterolemia: BioMed Research International Annual 2014.

Tiwari, U. and Cummins, E. (2010). Meta- analysis of the effect of beta-glucan intake on blood cholesterol and glucose levels. Institute of Food and Health, School of Agriculture, Food Science and Veterinary Medicine, University College Dublin, Belfield, Dublin, Ireland: Nutrition, 27 (2011), 1008–1016.

Comparative Study of the Efficacy of Fractional Erbium Glass Laser and Fractional Radiofrequency Microneedle in Treatment of Strriae Alba

Prapat Silawanna[1*] and Wipapen Chokdeesumrit[2]

[1]*Student in program Master of Science in Dermatology,*
School of Anti-aging and Regenerative Medicine, Mae Fah Luang University, Thailand
[*]*Corresponding author, Email : Emprapat@gmail.com*

Abstract

Background: Several studies have demonstrated that fractional Er:Glass laser is effective and safe in treatment of striae alba. A new technology fractional radiofrequency microneedle (FRM) is minimally invasive that its principle is penetration of RF to deeper thermal energy with minimal cutaneous burns, stimulating the production of new collagen and elastin. Until now, there is only one study of FRM device for treatment of striae distensae and no comparative study of these two modalities has been conducted in terms of safety and efficacy.
Objective: To compare efficacy and safety of fractional Er:Glass laser and FRM in treatment of striae alba.
Materials and Methods: Twenty volunteers with striae alba at both sides of abdomen, or buttock, or hip, or thigh were enrolled. All volunteers were received three treatments every 4 weeks. Each volunteer was randomly received both fractional Er:Glass laser and FRM treatment on both sides of the body (right and left). Clinical improvement in the appearance of striae alba was evaluated by two independent physicians at 4-week after the last treatment, using digital camera-photographs. Width, elasticity and surface smoothness of striae alba were measured by caliper, cutometer® MPA 580 and visioscan® VC 98, respectively. Side effects and volunteers' satisfaction were assessed by questionnaires.
Results: Twenty volunteers completed the study. Both sides were statistically significant in reduction of width and increasing in surface smoothness of striae alba, comparing baseline to the last follow-up. Global improvement scale showed no statistically significant difference between two groups. 75% and 82.5% of the subjects in fractional Er:Glass and FRM treatment respectively had better than 25% clinical improvement. Satisfaction score also showed no significant difference in both groups. FRM showed less side effects and shorter downtime than fractional Er:Glass laser.
Conclusions: Fractional radiofrequency microneedle was equally effective and safe to fractional Er:Glass laser in clinical improvement of striae alba. Satisfaction of outcomes showed no significantly difference between both treatments, and FRM had less side effects and shorter downtime. In conclusion, FRM appears to be a promising alternative for the treatment of striae alba.

***Keywords*:** Striae Alba/Fractional Er:Glass Laser/Fractional Radiofrequency Microneedle (FRM)

Introduction

Striae distensae are dermal scars with flattening and atrophy of the epidermis[1], which cause an irregular roughness of the skin. It is a relatively common skin condition which occurs in all races and more frequently in women than in men. Most common sites are buttock, thigh, abdomen, knee and breast[2]. Although etiologies are still obscure, presumed contributing factors are hereditary predisposition, mechanical stretching and rupture of connective tissue framework and hormonal changes. They are commonly found in adolescence, obesity and pregnancy and also Cushing's syndrome and long-term steroid use[3]. Even though striae distensae are not a huge health problem, they can cause a psychological problem to patients.

Up until now there is still no any effective modality that yields a consistent outcome in treatment of striae distensae. There are two main types of striae distensae, which are striae rubra and striae alba. Striae alba are permanent and similar to an atrophic scar[4], and more difficult to treat[5] therefore, it has been a challenge for dermatologists. Fractional photothermolysis is the most effective method for treatment of striae alba[6]. 1,550-nm fractional Er:Glass laser is as effective as 10,600-nm fractional CO2 laser, but it is more favorable due to shorter downtime and less side effects especially postinflammatory hyperpigmentation in darker skin type[7].

Radiofrequency (RF) has been reported to be effective and safe in treatment of striae alba. RF penetrates deeper thermal energy with minimal cutaneous injury, stimulating the production of new collagen and elastin[8]. Recently fractional radiofrequency microneedle (FRM) device, which is minimally invasive, has been introduced to use for large pores, skin tightening, acne vulgaris, acne scars[9] and also striae distensae[10].

Despite several studies have demonstrated the efficacy of fractional Er:Glass laser for treatment of striae distensae, up until now, there is only one study of FRM device for striae distensae, and no comparative study of these two modalities for striae distensae has been conducted in terms of safety and efficacy.

The purpose of this study was to compare the clinical efficacy and safety of fractional Er:Glass laser and FRM in treatment of striae alba.

Materials and Methods

All participants were informed of the study information and purpose, and inform consent was obtained prior to the study. The study was approved by the Ethical Committee on Research Involving Human Subjects, Mae Fah Luang University and performed in accordance with Declaration of Helsinki (1975). This randomized, single-blind clinical trial, within participant comparison study was done from December 2015 to March 2016. Twenty subjects, aged 23-40 years with skin phototype III (n = 5), and IV (n = 15) were recruited in this study. All subjects had striae alba on both sides of abdomen, or buttock, or hip, or thigh. Exclusion criteria were a history of keloid and photosensitivity, or usage of topical or systemic retinoids and glucocorticoids. Subjects who had received striae treatment during the prior 6 months were excluded, as who were pregnant or lactating. No other striae treatment was allowed during the study.

Treatment methods

All subjects were received three treatment sessions every 4 weeks. Each participant was randomly received both fractional Er:Glass laser and FRM treatment on his right and left side of the body. The same physician performed all treatments. Preoperatively topical EMLA cream was applied to the treatment areas under occlusion for one hour, and then sterilized them with 70% alcohol. Fractional Er:Glass laser was performed with Fraxel® Dual 1550/1927 (Solta Medical Inc., USA) on the parameter of 1550-nm, pulse energy 30 mJ, treatment coverage 3-4 and three passes. FRM utilizing INTRAcel® (Jeisys Medical Inc., Korea) was performed on the setting of bipolar mode, 1.5-2.0 mm in depth of needle (49 microneedles in 1 cm^2-electrode tip), energy level 2-3 and three passes. The treatment areas were closely observing that usually appeared erythematous and swollen within a few minutes. The parameter (treatment coverage or energy level) would be reduced if participants had symptoms of erythema longer than two weeks. After the treatment, a moisturizing cream was provided and avoiding irritation was recommended to the participants.

Treatment evaluations

Patient follow-up was scheduled at 4-week interval during 8-week treatment period and at 4-week interval after the final session (Total duration, 12 weeks). Evaluations including width, elasticity, surface smoothness of striae alba were obtained at baseline and every 4 weeks, and clinical photographs of the treatment area were taken at baseline and the final week. Side effects of treatment were recorded every session.

Subjective assessment

Digital photographs of clinical improvement in the appearance of striae alba were assessed on the last visit by two non-treating, masked physicians that was "Global improvement scale", using a quartile grading scale (1 = < 25%, 2 = 25-50%, 3 = 51-75%, 4 = > 75% improvement). Subjects were also requested to self-evaluate the treatment efficacy from the photographs (Satisfactory score; 0 = not satisfied, 1 = slightly satisfied, 2 = satisfied, 3 = very satisfied, 4 = extremely satisfied).

Objective assessment

The width was measured by a caliper at the widest of three striae in the treated area. The striae elasticity and surface smoothness were evaluated by using Cutometer® MPA 580 and Visioscan® VC 98, respectively.

Statistic for Data Analysis

Statistical Package for Social Sciences (SPSS version 19.0, SPSS Inc., Chicago, IL, USA) was used for all statistical analysis. Evaluation of width, elasticity and surface smoothness of striae alba at week 4, 8 and 12, Paired t-tests were used to compare with those of baseline, and Unpaired t-tests were used to compare those between two groups. Analysis of global improvement scale and satisfactory score, comparing between two groups, used Mann-Whitney U-test. And side effect data was analyzed by Mann-Whitney U-test and fisher exact test. The confidential level was set at 95%. Significance levels for all analyses were set at p-value < 0.05.

Results

Demographics

All twenty participants (age ranged 23-40 years, median age of 30.6 ± 4.77 years.), female (n = 17) and male (n = 3), completed the study. Most participants had Fitzpatrick's skin type IV (n = 15, 75%) and type III of the others. The most common site of striae alba was both sides of the buttock (n = 10, 50%).

Physician's global assessment

In the area treated with fractional Er:Glass laser, the clinical improvement of striae alba was evaluated as 27.5% of the subjects in grade 4 (> 75% improvement), and 47.5% of the subjects in grade 2 and grade 3 (25-75% improvement). In the FRM-treated group, it was evaluated as 70% of the subjects in grade 2 and grade 3 (25-75% improvement), and 12.5% of the subjects in grade 4 (> 75% improvement). The mean of global improvement scale was 2.55 in the fractional Er:Glass laser-treated group, and 2.4 in the FRM-treated group. There was not statistically significant difference in global improvement scale between two groups.

Participant's satisfaction assessment

60% of subjects in each group reported very satisfied and extremely satisfied, and only 5% of them in each group reported not satisfied to both fractional Er:Glass laser and FRM treatment. The means of satisfaction score were 2.65 which equally in both groups.

Width of striae

In fractional Er:Glass laser treatment, the average of width of striae (mean±SD) was 4.27±1.46 mm at baseline which significantly declined 8.20% and 13.58% at week 8 and week 12 ($p = 0.001$) respectively, compared with baseline. In FRM treatment, the width average (mean±SD) was 4.32±1.25 mm at baseline which significantly declined 2.55% and 7.64% at week 8 ($p = 0.008$) and week 12 ($p < 0.001$) respectively, compared with baseline. The difference comparing the decreased value of the width avarage (mean±SD) between two groups at baseline and 4 weeks after the last treatment was not significant.

Striae elasticity

In fractional Er:Glass laser treatment, there was only significant difference in the elasticity at baseline and week 12 ($p = 0.033$). In FRM treatment, there was no significant difference in the elasticity compared with baseline. The difference comparing the increased elasticity measurements (mean±SD) between two groups at baseline and 4 weeks after the last treatment was not significant.

Striae surface smoothness

Both treatments had significant differences in the smoothness at week 4, week 8, and week 12 ($p < 0.001$), compared with baseline. The difference comparing the increased smoothness measurements (mean±SD) between two groups at baseline and 4 weeks after the last treatment, 51.18% and 29.98% respectively, was significant ($p = 0.026$).

Side effects

Participants significantly experienced more pain, erythema and edema in fractional Er:Glass laser than FRM treatment.Postinflammatory hyperpigmentation, which occurred in both treatments, was also reported much more in fractional Er:Glass laser. Striae alba treated with FRM had shorter downtime, average 5 days comparing to 12 days in treatment of fractional Er:Glass laser. No other adverse events, such as blister or infection, were observed in any subject. Overall, FRM treatment showed less side effects and shorter downtime than fractional Er:Glass laser.

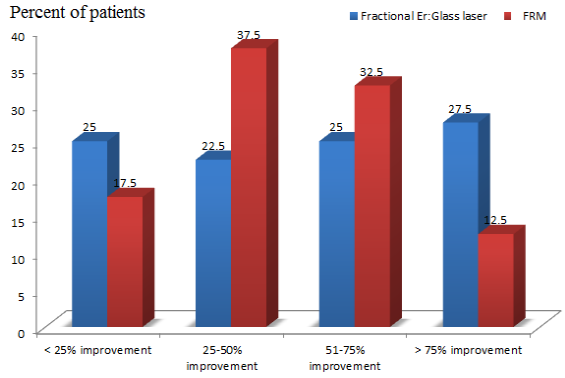


Figure 1. Evaluation of efficacy using physician's global improvement scale. The y-axis represents the percentage of patients, and the x-axis represents the clinical improvement in a quartile grading. The number of patients within each scale is shown.

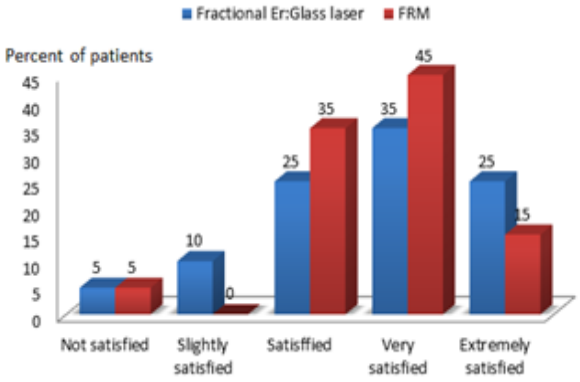
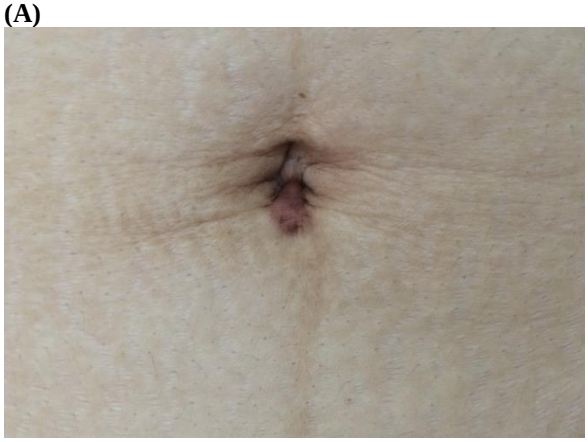


Figure 2. Evaluation of efficacy using patient's satisfactory score. The y-axis represents the percentage of patients, and the x-axis represents the satisfaction in a five-point grading. The number of patients within each scale is shown.

Discussion

Striae alba is difficult to prevent or treat and can cause significant cosmetic problems. There have been numerous challenges, but still there is no criterion standard in the treatment of striae distensae. A fractional radiofrequency microneedle device can deliver higher volumetric heating and deeper heat diffusion than lasers such that we can expect more-effective dermal remodeling. Furthermore, skin needling with microneedles has been reported to stimulate migration and proliferation of keratinocytes and fibroblasts by inducing the release of several growth factors[10].

(A)



(B)

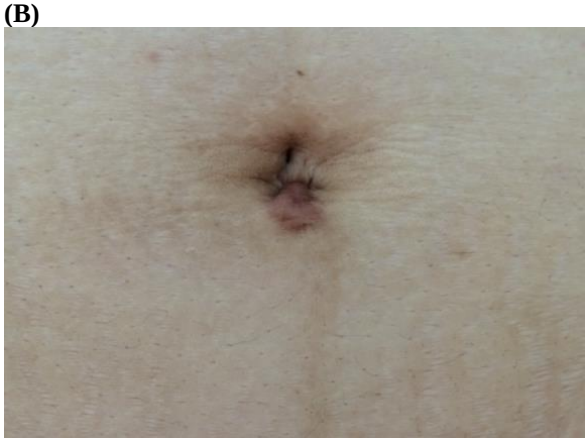


Figure 3. Striae alba in the abdomen : (A) Before treatment; (B) 4 weeks after three monthly treatments which fractional Er:Glass laser was performed on the right side, and FRM on the left side.

Although there has not been any research comparing efficacy of fractional Er:Glass laser and FRM in treatment of striae alba, the study of Ryu et al. showed the effective outcome of FRM for striae distensae, which was confirmed with our study. From the study, our data showed that 82.5% of the subjects in the FRM-treated group had better than 25% clinical improvement, comparing with 75% of the subjects in the fractional Er:Glass laser-treated group. The mean of global improvement scale was 2.4 and 2.55, respectively, which was not statistically significant. By objective assessments, fractional Er:Glass laser seemed to be better in improvement of width, elasticity and surface smoothness of striae alba than FRM. These could be resulted from treatment coverage area that fractional Er:Glass laser was more thorough than FRM. Previous studies on wound healing after FRM treatment demonstrated that radiofrequency thermal zones containing denatured collagen were maintained in the reticular dermis for longer than 28 days after treatment, although new dermal tissue partially replaced the zones. Hence, FRM treatment of striae alba produces slower developing improvement than fractional Er:Glass laser treatment[10]. Therefore, FRM parameter should be adjusted and longer follow-up period is suggested in further study.

In term of participants' satisfaction, it was similar to the physician evaluation of the outcome. There was no significant difference between both treatments. Side effects were also analyzed in this study. More participants complained of pain during fractional Er:Glass laser treatment. The pain rarely persisted, but could influence participant adherence. FRM could lead to only slight changes in epidermal barrier function resulting in less side effects and shorter downtime. It was an advantage of FRM comparing to fractional Er:Glass laser, which had to carefully operate due to postinflammatory hyperpigmentation events especially in darker skin type.

Conclusions

Fractional radiofrequency microneedle was equally effective and safe to fractional Er:Glass laser in clinical improvement of striae alba. Satisfaction of outcomes showed no significantly difference between both treatments, and FRM had less side effects and shorter downtime. In conclusion, FRM appears to be a promising alternative for the treatment of striae alba. Further study should be conducted to evaluate in longer follow-up period with more participants.

Acknowledgements

We would like to thank Dr.Pimsawat Watcharakan and Dr.Chinnawat Reanmanee for their assistance in the clinical assessment of the treatment outcome of the study subjects.

References

1. Tay, Y. K., Kwok, C. & Tan, E. (2006). Non ablative 1,450 nm diode laser treatment of striae distensae. Lasers Surg Med, 38, 196–99.
2. Cho, S., Park, E. S., Lee, D. H., Li, K. & Chung, J. H. (2006). Clinical features and risk factors for striae distensae in Korean adolescents. J Eur Acad Dermatol Venereol, 20, 1108–13.
3. Nigam, P. K. (1989). Striae cutis distensae. Int J Dermatol, 28, 426–428.
4. Elsaie, M. L., Baumann, L. S. & Elsaaiee, L. T. (2009). Striae distensae (stretch marks) and different modalities of therapy: an update. Dermatol Surg, 35, 563-73.
5. Alexiades-Armenakas, M. R., Dover, J. S. & Arndt, K. A. (2008). The spectrum of laser skin resurfacing: nonablative, fractional, and ablative laser resurfacing. J Am Acad Dermatol, 58, 719-37.
6. Savas, J. A., Ledon, J.A., Franca, K. & Nouri, K. (2014). Lasers and lights for the treatment of striae distensae. Lasers Med Sci, 29, 1735–1743.

7. Yang, Y. J. & Lee, G. Y. (2011). Treatment of striae distensae with nonablative fractional laser versus ablative CO2 fractional laser: a randomized controlled trial. Ann Dermatol, 23, 481–9.
8. Hruza, G., Taub, A. F., Collier, S. L. & Mulholland, S. R. (2009). Skin rejuvenation and wrinkle reduction using a fractional radiofrequency system. J Drugs Dermatol, 8, 259–65.
9. Lolis, M. S. & Goldberg, D. J. (2012). Radiofrequency in cosmetic dermatology: A Review. Dermatol Surg, 38, 1765–1776.
10. Ryu, H. W., Kim, S. A., Jung, H. R., Ryoo, Y. W., Lee, K. S. & Cho, J. W. (2013). Clinical improvement of striae distensae in Korean patients using a combination of fractionated microneedle radiofrequency and fractional carbon dioxide laser. Dermatol Surg, 39, 1452–8.

Comparison between Trunk Flexor and Extensor Endurance in Worker Characters

Haifah Samae[1], Wattana Jalayondeja[2*], Keerin Mekhora[3] and Chutima Jalayondeja[4]

[1] *Master degree student, Faculty of physical therapy, Mahidol University, Thailand.*
[2] *Assistance professor, Faculty of physical therapy, Mahidol University, Thailand.*
[3] *Lecturer, Faculty of physical therapy, Mahidol University, Thailand.*
[4] *Assistance professor, Faculty of physical therapy, Mahidol University, Thailand.*
** corresponding author. E-mail: wattana.jal@mahidol.ac.th*

Abstract

The present study investigated and compared trunk flexor and extensor endurance among three worker groups which are office, survey and manual material handling (MMH) workers. Sixteen male in office (mean age 29.69±3.09), eleven male in survey (mean age 28.36±4.11) and eighteen male in MMH (mean age 29.50±3.73) with no history of musculoskeletal, neurological and cardiovascular disorder were recruited from a petroleum exploration business. Trunk muscle endurance tests including trunk extensor flexor endurance and the ratio between flexor/extensor endurance were determined. The results showed no significant differences of both endurance times and its ratio among the 3 groups. However, office workers tend to have greater performance of trunk flexor endurance and trunk flexor/extensor endurance ratio but lower trunk extensor endurance times than other two groups. Based on the result, we suggested office workers have high possibility of low back pain than the other groups. This may cause by imbalance between trunk flexor and extensor endurance muscles and the low endurance of trunk extensor muscles.

Keyword: comparison, trunk flexor, back extensor, endurance and worker

Introduction

Low back pain (LBP) is one of major health problems among working population. Systematic reviews showed prevalence of low back pain ranged from 5% to 65% [1, 2]. Risk factors of low back pain are including lifting overload and prolong poor posture during working [2]. The self-reported musculoskeletal disorder study in Thai office workers found 34% complaint of low back pain [3]. Manual material handling worker is also affect from LBP by lifting task [4]. Work-related low back disorder leads to decrease worker performances and increase sick leave rate. For example in USA, LBP caused 33-41% of total compensation cost of work [2]. An imbalance between trunk flexion and trunk extension is a risk factor of low back pain.

Beiring-Sorensen test is generally used in an assessment of trunk extensor endurance [5, 6, 7]. Flexor endurance test is also used for measuring trunk flexor endurance [5, 6]. Both above isometric trunk endurance tests are applied to predict LBP [7, 8, 9]. Industrial workers affected from LBP performed less trunk extensor endurance performance when compared to trunk flexor [6]. Low isometric back endurance associated to high percentage of complaints of low back pain in men over one year period of a prospective study [8]. The assessment of trunk muscle endurance among workers is one of important aspects that investigate risk of LBP related to work and improve working capacity. Few studies have been measured trunk muscle endurance in Thai population [10, 11, 12]. No study compares trunk endurance performance among workers. Therefore, the current study determines assessment and comparison of trunk endurance among worker.

The objective of study

To compare trunk muscle endurance and its ratio among office, survey and manual material handling workers.

Hypothesis of study

There would be significant differences of trunk flexor, trunk extensor endurances and a flexor/extensor endurance ratio among three types of workers.

Materials and Methods

Procedure

Objectives of the study and procedure detail were explained before inform consents were signed. Healthy people with no musculoskeletal or neurological history of injuries were included. A questionnaire was used to collect demographic information and frequency of exercise. Blood pressure was measured before performing trunk endurance tests. Trunk extensor endurance was measured by modified Sorensen Test [12]. Participants were in prone lying on the test table with level of ASIS aligned with the edge of the table and the arms were crossed on the chest. Half of the lower body was strapped down to the table at 3 levels; ankles

at the level of the malleoli, knee creases and hips at the level of greater trochanters. The subject was asked to maintain horizontal position of trunk by touching a feedback pad in trunk extensor endurance test (figure 1). In trunk flexor endurance test, the participants inclined at 45 degree of trunk flexion by a prefabricated wedge. Both hips and knees were flexed and arms are crossed at chest level. The ankles were strapped with the test table. Then the prefabricated wedge was moved away, the participants were asked to hold in this inclined position by touching the feedback pad (figure 2). 10 minutes rest between tests. Due to the effect of gender on trunk endurance, only male workers were included. The current study was approved by Mahidol University Human Research Ethics Committee.

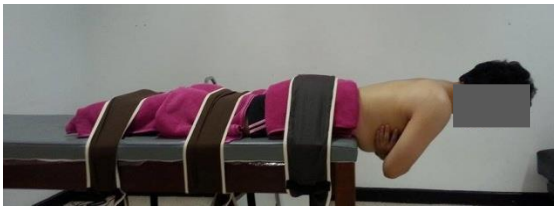


Figure 1. Trunk extensor endurance test

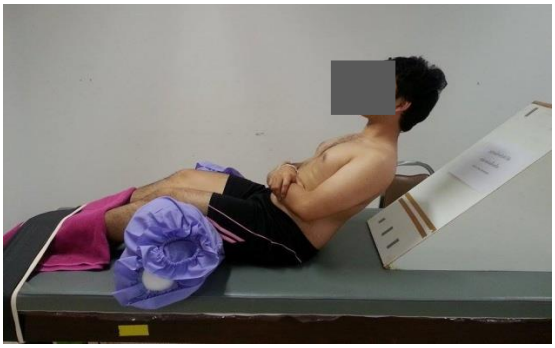


Figure 2. Trunk flexor endurance test

Study design

Cross-sectional study

Statistic analysis

SPSS version 19.0 was used for descriptive statistics (frequencies, mean and standard deviation) and statistical analysis. Normal distributions of the data were analyzed by Komogorov Smirnov Goodness of Fit test. One way-ANOVA and Kruskal-Wallis were used for parametric data and nonparametric data, respectively. The level of significance was set at 0.05.

Results and Discussion

Results

Sixteen participants were office workers. Their age range was between 26 to 35 years with the mean age of 29.69±3.09. Survey group (n =11) had age ranged from 21 to 34 years and the mean age was 28.36±4.11 years old. MMH group (n=18) had the age range of 23 to 35 years with the mean age of 29.50±3.73 years old. The results showed no significant differences of the mean of age, BMI, trunk endurance test among the three groups of workers. However, office workers seem to have greater trunk flexor endurance time and trunk flexor/ extensor endurance ratio but lower trunk extensor endurance while MMH workers had greater performance of trunk extensor endurance.

Table 1. Trunk endurance times in 3 kinds of workers

Variables	Mean (SD)			p-value
	Office (n=16)	survey (n=11)	MMH (n=18)	
Age[a] (years)	29.69 (3.09)	28.36 (4.11)	29.50 (3.73)	0.617
BMI[a](kg/m^2)	23.85 (2.85)	23.23 (2.14)	24.47 (3.64)	0.565
Extensor[a] endurance (sec)	81.30 (22.59)	93.93 (23.23)	95.70 (24.82)	0.185
Flexor[b] endurance (sec)	91.88 (61.80)	75.27 (32.27)	85.53 (35.56)	0.616
F/E ratio[a]	1.14 (0.65)	0.83 (0.38)	0.91 (0.35)	0.227

[a] One way-ANOVA, [b] Kruskal-Wallis test

Discussion

The study results are in contrasts to our hypothesis. It was shown that no significant differences of endurance times among workers were found. From our survey results, 66.67 % of office workers exercise regularly more than 3 times per week when compared to 58.82 % in MMH and 42.86 % in survey workers. Physical activity could promote muscle endurance by produce musclin as an exercise factor being secreted during vigorous exercise and daily routine of physical activities [15]. Exercise is one factor that can increase trunk muscle endurance of participants especially in the office workers. This may result in the findings with no significant differences of extensor and flexor endurance times among the 3 groups were found.

However office workers had trend to have low performance of trunk extensor endurance. Beiring-sorensen or isometric trunk extensor endurance test can predict the first time occurrence for low back pain during one year follow-up [8]. There was a confirmed study of Luoto et al. (1995) that only back muscle performance indicated risk factor of low back pain significantly [13]. Therefore, office workers may be at risk of low back pain more than the other workers group. Prolonged sitting is one of a cause of low back pain. Morl and Bradl (2013) studied lumbar posture and back muscle activation during 2 hours of sitting with support and unsupported backrest among office workers. They reported that the degree of lumbar spine flexion (reverse lordotic curve angle) associated with duration of sitting. Back muscle including longissimus and multifidus muscles had low activity (5-12% of normalized activation). Gaps of these muscle activities were also analyzed. It was found that longissimus and multifidus muscles were activated for only 60% of working time during sitting. Therefore, the load is transferred into passive structures such as ligament, bone and disc. This can lead to low extensor endurance in office group and also can induce low back pain [14]. The current study showed that the mean±SD of hours of sitting period among worker groups were 6.43±2.84 hours, 4.14±2.34 hours and 2.89±2.09 hours for office, survey and MMH workers, respectively. Therefore, office workers spend a long time in sitting posture. This might be the reason why trunk extensor endurance time of office workers had a trend to be lower than that of the other two groups. Beiring-sorensen reported that trunk extensor endurance time was longer than 198 seconds indicated free low back pain whereas the endurance time less than 176 seconds can predict low back pain within in the next year [8]. In addition, poor performance of the extensor endurance time less than 58 seconds associated with three times higher risk of low back pain compared to average performance participants. Mean value of trunk extensor endurance time from this current study was low when compared to the normative values of Alarannta el al., (1994). The extensor endurance times were 97 secs in male and 87 secs in female workers [16]. This may be due to effects of different ethnicity. However, McGill mentioned that weaker trunk extensor endurance than trunk flexor endurance associated with low back pain. The ratio of trunk flexor and trunk extensor should be less than or equal to 1.00 [17]. The result showed the ratio of trunk flexor/extensor endurance was 1.14(0.65) in office workers while these ratios in the other 2 types of workers were less than 1.00. Office workers might have an imbalance between trunk flexor and extensor endurance which can lead to the risk of back pain when compared to the survey and MMH workers. In order to prevent low back pain in office workers, trunk endurance exercise, especially for the extensor group should be suggested.

Age and BMI may affect muscle endurance. Young people had better extensor endurance time when compared to older groups [18]. Kurt Jorgensen and Tom Nicolaisen (1986) study showed that weight and height influenced performance of trunk extensor endurance [19]. This the current study showed no significance of age and BMI among the 3 types of workers; therefore, the influence of these factors was not found.

Conclusion

Office work is one of work character related to work-related low back pain which affected from imbalance of trunk muscle endurance. Trunk extensor endurance exercise should be considered in office workers.

Limitation

Number of participants in each were quite small. The results of this study can be used with cautions. Future studies with higher number of subjects are warranted.

Acknowledgments

I would like thank all participants of the Petroleum Exploration Company.

References

1. Dagenais, S., Caro, J. and Haldeman, S. (2008). A systematic review of low back pain cost of illness studies in the United States and internationally. Spine J, 8(1), 8-20.
1. Beeck, R.O. and Hermans, V. (2000). Research on work-related low back disorders: Office for Official Publications of the European Communities.
2. Janwantanakul, P., Pensri, P. Jiamjarasrangsri, V. and Sinsongsook, T. (2008). Prevalence of self- reported musculoskeletal symptoms among office workers. Occupational Medicine, 58(6), 436-8.
3. Randall, SB. and Jeter, G. (1997). A guide to manual materials handling and back safety: Division of Occupational Safety and Health, North Carolina Department of Labor.
4. Reiman, M.P., Krier, A.D., Nelson, J.A., Rogers, M.A., Stuke, Z.O. and Smith, B.S. (2012). Comparison of different trunk endurance testing methods in college-aged individuals. International journal of sports physical.
6. McGill, S., Grenier, S., Bluhm, M., Preuss, R., Brown, S. and Russell, C. (2003). Previous history of LBP with work loss is related to lingering deficits in biomechanical, physiological, personal, psychosocial and motor control characteristics. Ergonomics, 46(7), 731-46.
7. Moreau, C.E., Green, B.N., Johnson, C.D., and Moreau, S.R. (2001). Isometric back extension endurance tests: a review of the literature. Journal of manipulative and physiological therapeutics, 24(2), 110-22.
8. Biering-sorensen, F. (1984). Physical measurements as risk indicators for low-back trouble over a one-year period. Spine, 9(2):106-19.
9. Ito T., Shirado, O., Suzuki, H., Takahashi, M., Kaneda, K. and Strax, T.E. (1996). Lumbar trunk muscle endurance testing: an inexpensive alternative to a machine for evaluation. Archives of physical medicine and rehabilitation, 77(1), 75-9.
10. Taechasupamorn, T. Kongsawad, S. and Prapnakorn, L. (2002). Endurance times of trunk muscles in Thai elders aged 60-69 years. Journal of Gerontology and Geriatric Medicine, 2(4).
11. Piangchareon, N. (2001). Effects of trunk stabilization training in young sprinters of Chiang Mai running association (M.S. Thesis in Sport Science). Chiang Mai: Faculty of Graduated Studies, Chaing Mai university.
12. Kriangchieocharn, S. (2003). Trunk muscle endurance in Thai sedentary workers aged 20-49 years (M.S. Thesis in Physiotherapy). Faculty of Graduate studies, Mahidol University.
13. Luoto, S., Heliovaara, M., Hurri, H. and Alaranta, H. (1995). Static back endurance and the risk of low-back pain. Clinical biomechanics, 10(6), 323-4.
14. Morl, F., and Bradl, I. (2013). Lumbar posture and muscular activity while sitting during office work. Journal of Electromyography and kinesiology, 23(2), 362-8.
15. Subbotina, E., Sierra, A., Zhu, Z.et al. (2015). Musclin is an activity-stimulated myokine that enhances physical endurance. Proceedings of the National Academy of Sciences, 112(52), 16042-7.
16. Alaranta, H., Hurri, H., Heliovaara, M., Soukka, A. and Harju, R. (1994). Non-dynamometric trunk performance tests: reliability and normative data. Scand J Rehabil Med, 26(4), 211-5.
17. McGill, S. (2002). Low back disorder: evidence-based prevention and rehabilitation, 2nd edition. Human Kinetics.
18. Gibbons, L.E., Videman, T.and Battié, M.C. (1997). Determinants of isokinetic and psychophysical lifting strength and static back muscle endurance: a study of male monozygotic twins. Spine, 22(24), 2983-2990.
19. Jorgensen, K. and Nicolaisen, T. (1986). Two methods for determining trunk extensor endurance. European journal of applied physiology and occupational physiology, 55(6), 639-644.

Comparison of Botulinum Toxin Type A in Treatment of Contouring Trapezius Hypertrophy

Napamon Supornpun[1] and Paisal Rummaneethorn[2]

[1]Student in Master of Science Program in Dermatology,
School of Anti-Aging and Regenerative, Mae Fah Luang University,Bangkok 10220 Thailand
[2]Corresponding author: paisalrr@yahoo.com

Abstract

Asia women tend to prefer long elegant neck and shoulder. Oversized, masculine shoulder and scapular can cause psychological stress in women. Reduction of the trapezius muscle can make the neck longer and thinner which makes the collarbone stand out. Also, Trapezius myalgia, chronic pain from the upper trapezius muscle is the most frequent type of neck pain in occupational group.
Both condition whether trapezius hypertrophy or myalgia can be treated with Botulinum toxin type A.
New Botulinum toxin type A (Neuronox®) which produced from the same strain of Clostridium botulinum as onabotulinumtoxinA (Botox®)) is widely used in Asia may have the same efficacy as Botox® for treatment of trapezius hypertrophy and myalgia

Keyword: trapezius hypertrophy, New botulinumtoxin type A (Neuronox®), onabotulinumtoxinA (Botox®).

Introduction

Asia women tend to prefer long elegant neck and shoulder. Oversized, masculine shoulder and scapular can cause psychological stress in women. Reduction of the muscles of the shoulder makes the neck look longer & thinner and makes the collarbone stand out.

Neck/shoulder pain is widespread among office workers with intensive computer use (Jensen, 2003,Juul-Kristensen et al.,2004,IJmker et al., 2006). Trapezius myalgia – chronic pain from the upper trapezius muscle – is the most frequent type of neck pain in this occupational group (JuulKristensenet al., 2006).

New Botulinum toxin type A (Neuronox®) which produced from the same strain of Clostridium botulinum as onabotulinumtoxinA (Botox®) is widely used in Asia may have the same efficacy as Botox® for treatment of trapezius hypertrophy and myalgia. So, the relationship between the dose and clinical effects of Neuronox® compared to Botox® remains to be determined.

Material and Methods

Randomized, Double-blind Clinical Trial was performed. 20 Thai subjects age between 20-45 years old, with trapezius hypertrophy who want to Reduction of the muscles of the shoulder makes the neck to look longer & thinner were randomly assigned to treatment with the Botox® 40 units and the Neuronox® 40 units injected on each of trapezius which we design four point of injection for trapezius along the imaginary line from 7th cervical spine to acromion process and divide into three part then perform four point of injection in the middle part to avoid accessory nerve which align in the middle. Each follow-up visit at days 30 the patient was took a photograph and trapezius size was measured by researcher at the same point of measurement and was CT scan at day 30 to compare thickness of trapezius to day 0. The land mark of measurement point was at the imaginary line between horizontal line of T1 and vertical line of scapular blade.(figure1)

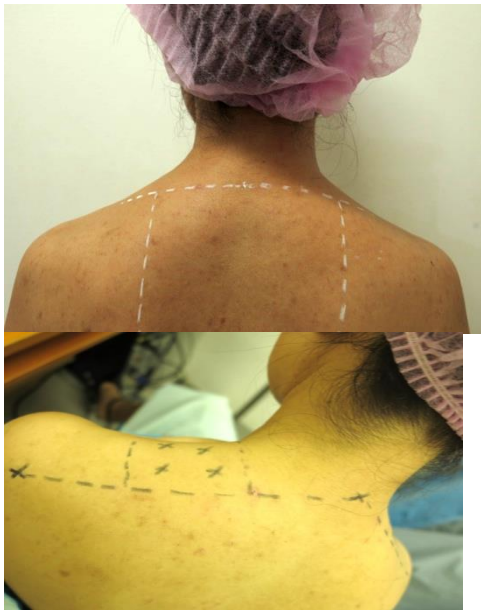


Figure 1 Point of measurement and Point of injection

Results and Discussion

Twenty volunteers completed the study. Thickness of trapezius was measured by CT scan and by researcher measurement

Result from CT scan trapezius thickness before Botox® injection was 1.6±0.35 cm. and after was 1.48±0.38 cm. and trapezius thickness before Neuronox® injection was 1.68±0.40 cm. and after was 1.35±0.35 cm .Paired differences of mean of trapezius thickness decrease after both botulinum toxin injection has no difference in significant level (P value<0.05) (figure2,3)

Result from researcher measure trapezius thickness before Botox® injection was 1.51±0.56 cm. and after was 0.82±0.45 cm. and trapezius thickness before Neuronox® injection was 1.65±0.60 cm. and after was 0.78±0.31 cm .Paired differences of mean of trapezius thickness decrease after both botulinum toxin injection has no difference in significant level (P value<0.05) (figure 4)

Pain score (0-10) of trapezius myalgia was decrease from 4.8±1.57 to 1.55±0.68 (figure6)And trapezius hypertrophy(0-10) concerning score was decrease from5.45±1.93 to 1.9±1.21

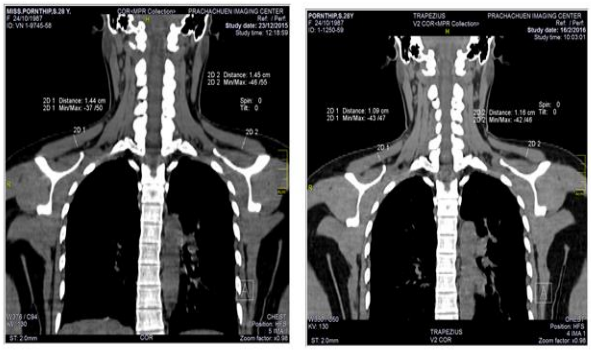


Figure 2 CT scan picture show trapezius muscle thickness decrease after botulinum toxin A injection

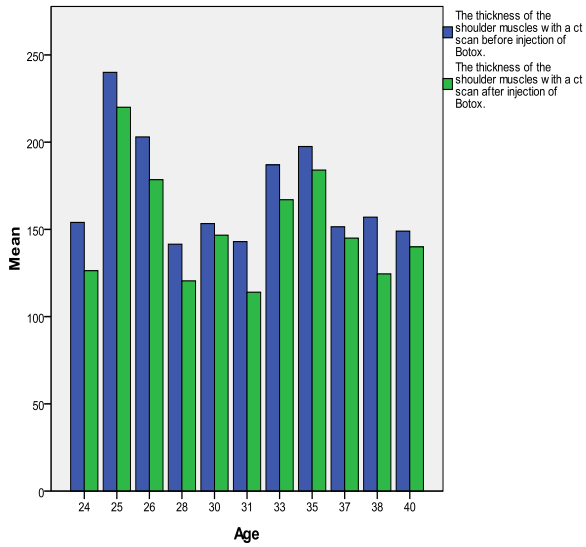
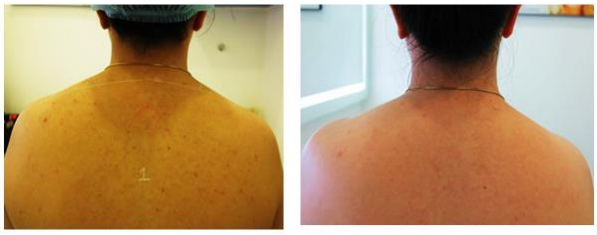


Figure 3 CT scan result analysis show trapezius muscle thickness decrease after Botulinum toxin type A injection



toxin A

Discussion

Botulinum toxin type A could be a useful treatment of trapezius hypertrophy and trapezius myalgia. Even though there is a good result of muscle reduction and pain relief after botulinum toxin type A injection in the same efficacy among Neuronox® and Botox®. But there are complications such as muscle pain, muscle weakness and bruising. 8 patients out of 20 (40%) mentioned of muscle pain which 5 of them were massager which use a lot of muscle power in every day. 7 patients out of 20 (35%) mentioned of muscle weakness. 1patients out of 20 (5%)mentioned of bruising. All of the complications have been relieved in 2 weeks.

Compare to Medytoxy Inc., trapezius muscle reduction injection guideline which suggested to apply 20 unit of botulinum toxin type A per side of trapezius muscle. In this study we apply 40 unit of botulinum

toxin type A in each side of trapezius muscle which was double dosage from this guideline .So, if the dosage was decreased to apply in this area, the complication might be less.

3 patient out of 20 (15%) whom suffered from trapezius myalgia mentioned of very satisfied of this treatment. 5 patients out of 20 (25%) were satisfied of their thinner and longer neck

In this study we have considered the proper point of Botulinum toxin type A in trapezius area which could be a proper guide of landmark of the injection.

Conclusion

From researched data analysis present that Botulinum toxin type A can treat trapezius hypertrophy and trapezius myalgia effectively and new Botulinumtoxin type A (Neuronox®) is equally effective in trapezius muscle reduction to onabotulinumtoxinA (Botox®). But both products also have complications such as bruising, muscle pain and muscle weakness.

Suggestion

This study has short period to follow up in only 1 month .For benefit of long term effect, further investigation should arrange in longer period.

Less Botulinum toxin type A dosage which apply in this area should be less than 40 unit per side to decrease side effect severity.

Measurement of trapezius thickness by CT scan was more precise than measurement point from picture of researcher due to there is no fat component containing to make the outcome error.

References

MEE YOUNG PARK,MD, KI YOUNG AHN,MD,PhD, AND DUCK SOO JUNG,MD,PhD(2003) Botulinum toxin type A for contouring the lower face.Dermatol surg 2003.May 29(5):477/83

Lars L. Andersen, Professor, DrMedSci, Gisela Sjøgaard(2008).;physical exercise in rehabilitation of trapezius myalgia; National Research Centre for the Working Environment, Copenhagen, Denmark.

Eng-King Tan;Yew-Long Lo Department of Neurology; John Thomas Department of Neurosurgery; Ling-Ling Chan Department of Radiology Singapore General HospitalNational Neuroscience Institute Singapore., CMAJ • June 5, 2007 • 176(12) | 1702; 2007 Canadian Medical Association**.**

Adler, S., Bicker, G., Bigalke, H., Bishop, C., Blumel, J., Dressler, D., Fitzgerald, J., Gessler, F., Heuschen, H., Kegel, B., Luch, A., Milne, C., Pickett, A., Ratsch, H., Ruhdel, I., Sesardic, D., Stephens, M., Stiens, G., Thornton, P., Thurmer, R., Vey, M., Spielmann, H., Grune, B. & Liebsch, M. (2010). The current scientific and legal status of alternative methods to the LD50 test for botulinum neurotoxin potency testing. The report and recommendations of a ZEBET Expert Meeting. Altern Lab Anim, 38(4), 315-330.

Benedetto, A. V. (2011). Botulinum toxins in clinical aesthetic practice. London: Informa Healthcare. Carruthers, A., & Carruthers, J. (1997). Cosmetic uses of botulinum A xotoxin. Adv Dermatol, 12, 325-347; discussion 348.

Comparison of Curcumin and Erythromycin on Acne Improvement

Ornkamol Inkong-ngam [1] and Paisal Rummaneethorn [2]*

Master of Science Program in Anti Aging and Regenerative Medicine,
[1,2]School of Anti-Aging and Regenerative Medicine, Mae Fah Luang University
38/11-13 Asoke Place Building, Asoke road, Sukhumvit 21,
Klong Toey Nua, Wattana, Bangkok 10110, Thailand
**Corresponding author Tel.:02-6644360, Fax. 02-6644362, E-mail paisalrr@yahoo.com*

Abstract

Acne is one of the most common skin problems in adolescents and middle ages people. Although acne usually resolves spontaneously but long-term complications such as hypertrophic/hypotrophic scars sometimes occur. Long-term use of oral antibiotics increases incidence of drug allergy and drug resistance. Curcumin extract is known for its anti-inflammatory and antioxidation properties. Previous in-vitro studies have shown that Curcumin extract has an inhibitory effect against *P.acnes*, the most common bacteria causing inflammatory acne. This study aimed to compare the effectiveness of Curcumin extract 2,000 mg/day with Erythromycin 1,000 mg /day for mild to moderate acne treatment. Forty-four patients with mild to moderate acne were enrolled in this prospective randomized double blind, controlled trial. Groups received either curcumin extract capsules 2,000mg/day or erythromycin 1,000 mg/day for eight-week duration. Changes in sebum measured by using a Sebumeter, Acne count, drug side effects, and satisfaction were evaluated at 4th and 8th week. The patients who prescribed with 2,000 mg/day of Curcumin had a significant decrease in sebum level compared to those prescribed with erythromycin, decreased by 22.83% vs. 8.36% respectively, at p= 0.014 . Inflammatory acne counts of the Curcumin group significantly decreased both in the 4th and 8th week (p= 0.003 and p = 0.008, respectively). However, in the 8th week, the effect on inflammatory acne was still lower than in those with erythromycin (18.98% vs. 35.04%, respectively). There was no significant difference in non-inflammatory acne count, total acne count, adverse side effect, and satisfaction between two groups. In the conclusion, 2,000 mg/day of Curcumin is effective to decrease sebum level and inflammatory acne. However, the effect on inflammatory acne is lower than Erythromycin.

Keywords: Curcumin/Curcuminoid/erythromycin/acne

Introduction

Acne is one of the most common skin problems that affects over 80% of teenagers and may persist beyond the age of 25. Typical lesions of acne include comedone, inflammatory papules, and pustules. Nodules and cysts could be found in more severe acne. Although acne usually resolves spontaneously but long-term complications such as hypertrophic/hypotrophic scars sometimes occur. Acne is caused by several etiologies: Follicular epidermal hyperproliferation and excess sebum production forming comedone then superimposed by *Propionibacterium acnes* infection and eventually lead to inflammation [1-3].

Most acne treatments focus on the causes by improving keratinization, decreasing sebum production, inhibiting P. acnes, and decreasing inflammation. Choices of treatment depend on the severities of acne. Topical agents are used in mild severity. In more severe acne, combined treatments of topical and oral medication such as antibiotics or oral isotretinoin are usually prescribed [4]. Oral Antibiotics inhibit P. acnes growth and accumulation, also reduce chemotaxis which are helpful in inflammatory acne consists of papules and pustules. Oral antibiotics such as Tetracycline hydrochloride 500-1000 mg/day, Doxycycline 100-200 mg/day and Erythromycin 1,000 mg/day are commonly prescribed. In case of Gram negative folliculitis is suspected, Sulfamethoxazole-Trimethoprim is considered as drug of choice despite of its severed side effects and drug allergy. Long-term use of oral antibiotics increases incidence of drug allergy and drug resistance. Therefore, new antimicrobial agents would be helpful for acne therapy.

Curcumin, a phytochemical substance, is extracted from *Curcuma longa* L. (Zingiberaceae family). It is also known as Turmeric and worldwide used as spice and herbal medicine. Curcumin has well-known properties such as anti-inflammatory, antioxidation, and wound healing. [5] In Thailand, 2,000-4,000 mg/day of Curcumin extract is prescribed for treatment of dyspepsia and has been enlisted in national list of essential drugs since 1999. Nowadays, there are several studies determining the biological activities of Curcumin including anticancer and antibacterial effect. Previous in-vitro studies have shown that Curcumin extract has an inhibitory effect against *P.acnes*, the most

common bacteria causing inflammatory acne [6]. This study aimed to compare the effectiveness of 2,000 mg/day oral curcumin with 1,000 mg/day erythromycin for mild to moderate acne treatment. The effect of curcumin and erythromycin on sebum level, drug side effect, and satisfaction were also evaluated.

Materials and Methods

Both male and female, all skin type and aged between 20 to 50 years-old volunteers who had mild to moderate acne were included. Those who had been recently received acne treatment (topical drugs or creams less than 2 weeks , oral antibiotics less than 4 weeks and oral isotretinoin less than 6 months) , had been taken oral curcumin extract and stopped less than 3 months , were pregnant or breastfeeding and who had contraindication for curcumin and erythromycin ,were excluded. Informed consent was obtained prior to the study.

Forty-four participants with mild to moderate acne were enrolled in prospective randomized double blind, controlled trial. Personal data, photographs, sebum levels measured by sebumeter and acne count were collected, and assessed by physician whom blinded to that study in order to avoid bias data measurements. Participant randomized group assignment was done by computerized program. Participants in each group were prescribed with 500 mg/capsule of curcumin or 250 mg/capsule of erythromycin, four times daily for eight-week duration , and received facial cleanser from researchers. Changes in sebum measured by using a Sebumeter, Acne count, drug side effects, and satisfaction were evaluated at 4th and 8th week.

Statistical analysis

Qualitative data such as sex, occupation, history of antibiotics use, satisfaction survey, acne severity, and drug side effects were presented in frequency and percentage. On the other hand, quantitative data such as age, duration of disease, sebum level and acne count were displayed in mean average and standard deviation (SD). Two-way, repeated measures ANOVA with Bonferroni method were used to compare sebum level before-after treatment, within and between groups (curcumin and erythromycin). Mean average acne counts (non-inflammatory, inflammatory, and total) were compared before-after treatment within group by using Wilcoxon sign rank test. Changes in mean acne count and satisfaction score between groups were compared by Mann-Whitney U-test. Side effects were compared between groups by Chi-square test. All statistical tests were performed with a significant level of 0.05.

Results

Of the 44 participants, 41 completed the 8-week trial period. Three participants (1 from curcumin group and 2 from erythromycin group) experienced drug side effect and decided to leave the study at 4th week. Baseline characteristics of both groups had no statistically difference. Most of participants were female, 25 year old, factory worker, 1 to 2-year duration of mild to moderate acne and never received antibiotics for acne treatment (Table 1). Both groups had no difference in sebum level and acne counts before starting the study (Table 2).

To compare the sebum level before and after treatment (Table 3) , curcumin group had significant decrease in sebum level from 80.4 ± 49.22 µg/cm^2 at the beginning to 60.40 ± 16.59 µg/cm^2 at the 4th week and to 53.99 ± 13.93 µg/cm^2 at the 8th week (p-value 0.021 and 0.003, respectively). On the other hand, the erythromycin group had slightly non-significant decrease in sebum level. When compared between groups, participants receiving curcumin had lower sebum level than those receiving erythromycin significantly at the 8th week (53.99 ± 13.93 µg/cm^2 vs. 64.32 ± 13.83 µg/cm^2 respectively, at p-value 0.002). The curcumin group also had greater change in sebum level than the erythromycin group at the 8th week (22.83% vs. 8.36% respectively, p-value 0.014)

Table 1: Baseline characteristics classified by curcumin and erythromycin group

Characteristics	Curcumin (n=22)		Erythromycin(n=22)		p-value
	Number	Percent	Number	Percent	
Sex					0.472[A]
female	18	81.8	16	72.7	
male	4	18.2	6	27.3	
Age (year)					
Mean±SD (min-max)	25.18±4.25(20-34)		24.50±4.58(20-35)		0.682[B]
Department					
factory	13	59.1	18	81.8	
office	9	40.9	4	18.2	
History of antibiotic use					0.304[C]
no	17	77.3	16	72.7	
topical	4	18.2	3	13.6	
topical and oral	1	4.5	3	13.6	
Duration of disease					
Mean±SD (min-max)	2.56±1.99(0.08-7)		2.41±2.29(0.2-10)		0.816[D]
<1 year	4	18.2	4	18.2	
1-2 year	8	36.4	11	50.0	
3-4 year	6	27.3	3	13.6	
5 year +	4	18.2	4	18.2	
Severity					0.761 [A]
mild	9	40.9	10	45.5	
moderate	13	59.1	12	54.5	

A, B, C, and D represented p-value from chi-square, student t-test, Fisher's exact and Mann-Whitney u-test, respectively

Table 2: Comparison of sebum level and acne count at baseline between curcumin and erythromycin group

At baseline	Curcumin (n=22)		Erythromycin(n=22)		p-value
	Mean	SD	Mean	SD	
Sebum level	78.81	48.61	72.43	18.78	0.569[A]
Acne count (lesion)					
Non-inflammatory	26.18	16.53	22.23	12.96	0.353[B]
Inflammatory	11.32	5.51	12.73	7.85	0.481 [B]
Total	37.50	19.92	34.86	17.82	0.496 [B]

A and B represented p-value from student t-test and Mann-Whitney u-test, respectively

Table 3 Comparison of sebum level between group (curcumin and erythromycin) and within group

Sebum level (µg/cm^2)	Curcumin (n=21)		Erythromycin(n=20)		p-value between group[A]
	Mean	SD	Mean	SD	
Baseline	80.40	49.22	71.41	18.00	0.447[A]
Week 4	60.40	16.59	67.57	17.71	0.188[A]
Week 8	53.99	13.93	64.32	13.83	0.002*[A]
Change (week4-baseline)	-20.01	44.41	-3.84	7.86	0.072 [B]
%Change of week4	-13.91	26.40	-4.86	8.90	0.098 [B]
Change (week8-baseline)	-26.41	46.29	-7.09	10.44	0.015* [B]
%Change of week 8	-22.83	21.95	-8.36	11.87	0.014* [B]
p-value within group [A]					
Baseline VS week 4	0.021*		1.00		
Baseline VS week 8	0.003*		1.00		

A and B represented p-value from repeated measure ANOVA and Mann-Whitney U-test, * =significant at p<0.05

To compare acne counts before and after treatment, both curcumin and erythromycin had significant effect on decreasing inflammatory acne count at the 4th and 8th week (Table 4) but had no significant change in non-inflammatory and total acne count. At the 8th week, the participants in curcumin group had less change in inflammatory acne counts than those in erythromycin group (18.98% vs. 35.04% respectively, p-value 0.015). Therefore, the curcumin group had significant higher inflammatory acne counts than the erythromycin group at the same week (8.29 ± 4.16 lesions vs. 5.55 ± 3.87 lesions, respectively; p-value 0.047). The common side effects of curcumin are diarrhea, nausea, vomiting, and dizziness. In this study, three participants in curcumin group had mild nausea symptoms and continued the drug until the end of treatment. Only one participant had diarrhea symptom that disturbed her job so she decided to leave the study. In the erythromycin group, mild nausea occurred to five participants. Two participants whom received erythromycin had severe nausea and vomiting, so they asked to discontinue. However, when statistically compared, there was no significantly difference of the side effect between two groups.

The satisfaction surveys were evaluated at the follow-up and found no statistically different between curcumin and erythromycin groups. Participants in both groups felt average to high satisfaction.

Table 4: Comparison of inflammatory acne count between groups (curcumin and erythromycin) and within group

Inflammatory acne count (lesion)	**Curcumin (n=21)**		**Erythromycin(n=20)**		**p-value between group[A]**
	Mean	**SD**	**Mean**	**SD**	
Baseline	11.19	5.61	11.90	7.41	0.948
Week 4	8.62	4.09	7.85	4.86	0.417
Week 8	8.29	4.16	5.55	3.87	0.031*
Change (week4-baseline)	-2.57	3.47	-4.05	5.31	0.260
%Change of week4	-17.62	26.07	-19.32	57.54	0.133
Change (week8-baseline)	-2.90	3.96	-6.35	6.44	0.047*
%Change of week 8	-18.98	37.84	-35.04	75.77	0.015*
p-value within group[B]					
Baseline VS week 4	0.003*		0.004*		
Baseline VS week 8	0.008*		0.001*		

A and B represented p-value from Mann-Whitney u-test and Wilcoxon Signed Ranks test

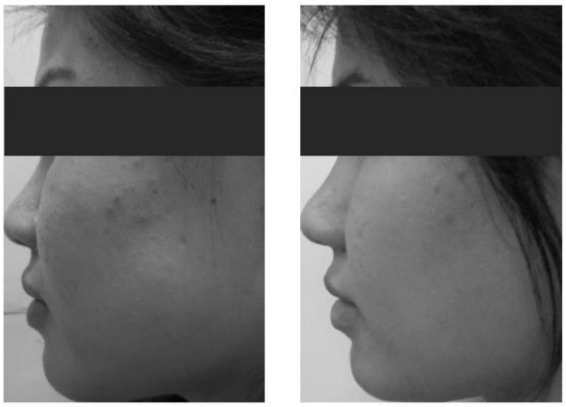


Figure 1. (a) Before treatment (b) After 8 weeks of treatment with oral curcumin, with decrease in acne lesion

Discussion

Sebum overproduction, *P.acnes*, and inflammation are major factors that aggravate acne formation. Curcumin has been proven that it can decrease sebum production and inflammation. Previous In-vitro studies have shown that curcumin had inhibitory effects on *P.acnes* [5,7]. In 13 male participants, split face controlled trial had shown that 5% curcumin extract cream significantly decreased sebum level by 3%, 14%, and 24.8% at the 4th, 8th, and 12th week, respectively [8]. In another clinical study, 30 breast cancer patients with radiation dermatitis received 6 grams per day of oral curcumin throughout their radiation courses, had less inflamed skin and lower severity score of dermatitis than those with placebo [9]. The effects showed significant changes since the 4th week. Consistently, this study has shown the significant changes in sebum level and inflammatory acne count starting from the 4th week.

Acne is one of the most common skin problems in Thais. Despites of spontaneously resolved, the complications such as hyper- or hypopigmentation and acne scars often occur. The disease and complications cause psychological lack of confidence and sociability of patients especially in adolescent. Long-term uses and high dosages of antibiotics lead to drug adverse reaction, drug allergy, and bacterial resistance. Many studies tried to find new antimicrobial agents that effectively treat acne and cause fewer side effect and drug resistance. Curcumin is phytochemical which has potency to inhibit numerous bacterial strains and has anti-inflammatory effect. This study shows that curcumin effectively improve acne symptoms by decreasing sebum level and inflammation with equal side effect and patients' satisfaction. Curcumin has several properties, which benefits acne symptoms and other health conditions. However, further studies should be conducted to evaluate efficacy of curcumin at different doses, longer study period, others acne severity, and comparing with other common-used antibiotics.

Conclusion

2,000 mg/day of Curcumin for 8 weeks is effective to decrease sebum level and inflammatory acne. The effect on inflammatory acne is lower than Erythromycin. With less side effect and same satisfaction score as erythromycin, therefore, curcumin could be considered as alternative drug choice for treating mild to moderate acne.

References

Jeremy A et al.(2003) .Inflammatory events are involved in acne lesion initiation. J Invest Dermatol, 121 ,20-27.

Downing DT, Stewart ME, Wertz PW, Strauss J. (1986). Essential fatty acids and acne. J Am Acad Dermatol , 14, 221-225.

Pochi PE and Strauss JS. (2003). Sebaceous gland response in man to the administration of testosterone, D-4androstenedione and dehydroisoandrosterone. J Invest Dermatol , 52, 32-36.

Fisher FJ , Voohess JJ. (1996). Molecular mechanisms of retinoid actions in skin. FASEB J , 10, 1012-1013.

Gopinath D, Ahmed MR, Gomathi K, Chitra K, Sehgal PK, Jayakumar R. (2004). Dermal wound healing processes with curcumin incorporated collagen films. Biomaterials , 25(10), 1911-1917.

Chi-Hsien Liu and Hsin-Ying Huang. (2013). In Vitro Anti-Propionibacterium Activity by Curcumin Containing. Chem. Pharm. Bull. ,61 (4), 419-425.

Nusavadee Pojananaukij and Somjai Kajorncheappunngam. (2010). Comparison of antimicrobial activity of Mangosteen crude, Tumeric and Gotu Kola extract. Naresuan University Journal , 18(1).

Shahiq uz Zaman and Naveed Akhtar (2013). Effect of Turmeric (Curcuma longa Zingiberaceae) Extract Cream on Human Skin Sebum Secretion. Tropical Journal of Pharmaceutical Research, 12 (5), 665-669.

Julie L. Ryana et al . (2013). Curcumin for Radiation Dermatitis: A Randomized, Double-Blind, Placebo-Controlled Clinical Trial of Thirty Breast CancerPatients. Radiat Res. , 180 (1), 34–43

Comparison of Oral Silymarin and Placebo on Melasma Treatment

Sirem-on Thienthai [1] and Thamthiwat Nararatwanchai [2]*

Master of Science Program in Anti Aging and Regenerative Medicine,
[1,2]School of Anti-Aging and Regenerative Medicine, Mae Fah Luang University
38/11-13 Asoke Place Building, Asoke road, Sukhumvit 21,
Klong Toey Nua, Wattana, Bangkok 10110, Thailand
**Corresponding author Tel.:02-6644360, Fax. 02-6644362, E-mail pitipalungwachira@hotmail.com*

Abstract

Melasma is a skin problem characterized by gray to brown symmetrical patches on sun-exposed areas of the skin such as cheek , forehead and upper lip. It is commonly found in hot climate country; Thailand. There are several treatment modalities but does not show satisfying outcomes. New researches are focused on antioxidant effect in melasma treatment which based on newly developed melasma hypothesis about oxidative stimulating effect from UV. Silymarin is an effective antioxidant found in Milk Thistle which can significantly inhibit tyrosinase enzyme, reduce melanin pigment and anti-inflammatory. Yet, no clinical study has been conducted regarding the potential benefits of oral Silymarin in the treatment of melasma. To compare effectiveness of oral Silymarin 1,260 mg./day with placebo on melasma treatment. Twenty-four participants with facial epidermal melasma were enrolled in prospective randomized double-blind, placebo-control trial. Participants in each group were prescribed with tablets of oral Silymarin or placebo for six weeks and followed up in 0, 3rd and 6th week. The outcome consisted of the evaluation of MASI score , mean melanin index (measured by Mexameter MX18) and patients self-evaluation and side effect. Within-groups comparison, when compare with baseline, only in placebo group has statistical significance, MASI score were significantly reduced only in the 3rd week of (p-value = 0.03) and mean melanin index significantly reduced only in the 6th week of participation (p-value = 0.02). Between-groups comparison, there were no statistical difference in MASI score and mean melanin index at any time of evaluation. Three participants from Silymarin group lost to follow up and one participant from placebo group developed skin rash at the inner arms and withdrew from the study. Oral Silymarin 1,260 mg./day for six-week period has no significantly effectiveness in melasma treatment when compared with placebo. However there was no serious side effect in Silymarin group.

Keyword: Silymarin/Milk Thistle/Melasma/Antioxidant

Introduction

Melasma is a common skin problem in hot climate country such as Thailand. Clinical characteristic is gray to brown patches at sun exposed area of the face eg. cheek , forehead and upper lip. The aggravating factors of melasma are Ultra Violet light (UV light) , hormone , pregnancy , some cosmetic and medication. The main factor is UV light and light [1]. Several researches show that UV light promotes melanocytes proliferation and melanin synthesis, which the pathway depends on the activity of Tyrosinase enzyme. Moreover UV light also leads to reactive oxygen species in epidermis which can be consider as another cause of melasma [2].

There are several treatment modalities eg. topical agents , peeling agents , laser and medication, but do not show satisfying outcomes. Recently researches are focused on antioxidant effect in melasma treatment which based on newly developed melasma hypothesis about oxidative stimulating effect from UV. From the results, oral antioxidant might be another potential treatment modality in term of reducing side effects from classical therapy.

According to previous review literatures, Silymarin which is derived from Milk Thistle *Silybum marianum (L.)* is a natural polyphenolic flavonoid. Its main component silybin (silibinin), is considered to be the most biologically active with anti-oxidant properties [3]. Silymarin and Silybin show cutaneous photoprotection effects by their ability to reduce and suppress harmful effect of solar UV radiation, such as UV-induced oxidative stress, inflammation, immune responses, DNA damage and apoptosis [4]. Silymarin significantly prevented melanin production in a dose-dependent manner with an IC50 value of 28.2 mcg./ml., without effect on cell viability [5]. and does not show any toxic or

teratogenic effect [6,7,8] . This study aimed to compare the effectiveness of 1,260 mg./day oral Silymarin with placebo for melasma treatment. The effect of Silymarin and placebo on MASI score, mean melanin index, drug side effect, and satisfaction were also evaluated.

Materials and Methods

Inclusion criteria were participants who diagnosed epidermal type melasma by Wood's lamp, 18-65 year of age and signed a written inform consent. While an exclusion criteria were participants who have history of allergy to Silymarin, previous melasma treatment within 4 weeks, pregnant and breastfeeding woman.

Twenty-four participants were enrolled in the study. History was taken from each particiant, photo was taken, MASI score was evaluated, mean melanin index was examined by MexameterMX18.

Participants were divided into 2 groups by computerized and block randomization. Each group were randomized and double-blind to receive the treatment ; Group 1 received placebo 9 tabs/day (3 tabs x 3 times daily), Group 2 received Silymarin (140 mg./tablet) 9 tabs/day (3 tabs x 3 times daily), both received moisturizer and sunscreen spf50 every visit. All participants were advised to avoid sun exposure and used sunscreen for the whole treatment period.

Participants were followed up every 3 weeks until 6 weeks period for assessment. MASI score were reevaluated, mean melanin index were reexamined, photo was taken every visit. Medication side effects and participants self-evaluation were recorded.

Statistical analysis

Qualitative data such as sex, occupation, previous treatment, adverse effect and self-evaluation were presented in percent and frequency. Quantitative data such as MASI score and mean melanin index were displayed in mean average and Standard Deviation (SD). Two-way, repeated measures ANOVA with Bonferroni method were used to compare mean melanin index before-after treatment, within and between groups (Silymarin and placebo). Student t-test was used to compare change in mean melanin index and percent of change in mean melanin index between groups. Mann-Whitney U-test was used to compare MASI score in 0 , 3rd and 6th week between groups, and used Wilcoxson sign rank test to compare MASI score before-after treatment within groups. Side effects were compared between groups by Fisher's exact test. All statistical tests were performed with a significant level of 0.05.

Results

Of the 24 participants, 20 completed the 6-week trial period. Three participants from Silymarin group lost to follow up and one participant in placebo group developed skin rash after took the medicine and withdrew from the study. Baseline characteristics of both groups had no statistically difference. Most of participants were female, mean age in Placebo group is 34.75 +/- 7.61 year and in Silymarin group is 36.5 +/- 10.11 year, 23 of participants are an employee and the other one is business owner, mean duration of disease in placebo group is 5.12 +/- 5.38 and in Silymarin group is 2.75 +/- 3.14, more than 50% in both groups never received any treatment. Mean melanin index and MASI score at baseline have no statistical difference (p-value 0.93 and 0.91, respectively). (Table 1 and 2)

Table 1 Baseline characteristics classified by placebo and Silymarin group.

Characteristics		Placebo(n=12)		Silymarin (n=12)		p-value
		Number	Percent	Number	Percent	
Sex						1.00[A]
	Male	2	16.7	1	8.3	
	Female	10	83.3	11	91.7	
Age (yr)						
	Mean+SD (min-max)	34.75±7.61 (21-48)		36.50±10.11 (22-51)		0.64[B]
Occupation						1.00 [A]
	Business owner	1	8.3	-	-	
	Employee	11	91.7	12	100	
Duration of disease						
	Mean+SD (min-max)	5.12±5.38 (1-20)		2.75±3.14 (1-12)		0.20[C]
Cause of disease						0.68 [A]
	Unknown	7	58.3	5	41.7	
	UV light	5	41.7	6	50	
	Pregnancy	-	-	1	8.3	
Previous treatment received						1.00 [A]
	None	7	58.3	7	58.3	
	Medical treatment	1	8.3	1	8.3	
	Cosmetic care	4	33.3	4	33.3	

A,B, and C represented p-value from Fisher's exact test, student t-test, and Mann-Whitney u-test, respectively

Table 2 Comparison of Mean melanin index and MASI score at baseline between placebo and Silymarin group.

At baseline (week 0)	n	Mean	SD	Median	Min	Max	p-value
Mean melanin index							0.93 [A]
Placebo (n=11)	12	292.69	54.72	286.3	210.0	389.0	
Silymarin (n=9)	12	294.64	49.11	292.9	222.8	428.7	
MASI Score							0.91[B]
Placebo (n=11)	12	12.03	4.11	12.4	6.6	17.1	
Silymarin (n=9)	12	12.77	6.99	10.4	4.8	26	

A and B represented p-value from student t-test and Mann-Whitney u-test

Within-groups comparison, when compare with baseline, only in placebo group has statistical significance, MASI score were significantly reduced only in the 3rd week, from 12.03 +/- 4.11 to 10.12 +/- 3.16 (p-value = 0.03) and mean melanin index were significantly reduced only in the 6th week, from 294.57 +/- 56.98 to 268.83 +/- 64.13 (p-value = 0.02). Between-groups comparison, there were no statistical difference in MASI score and mean melanin index at any time of evaluation. Mean melanin index in placebo group were 294.57 ± 56.98, 276.39 ± 61.07 and 268.83 +/- 64.13 at baseline, the 3rd and the 6th week, respectively. While in Silynarin group were 279.44 +/- 28.43, 271.46 +/- 35.40 and 269.28 +/- 31.38 at baseline, the 3rd and the 6th week, respectively. MASI score in placebo group were 12.03 +/- 4.11, 10.12 +/- 3.16 and 9.95 +/- 2.68 at baseline, the 3rd and the 6th week, respectively. While in Silymarin group were 12.77 +/- 6.99, 11.0 +/- 5.34 and 11.27 +/- 4.76 at baseline, the 3rd and the 6th week, respectively. (Table 3 and 4)

Table 3 Comparison of mean melanin index between and within placebo and Silymarin group.

Mean melanin index	Placebo (n=11)		Silymarin (n=9)		p-value between group A
	Mean ±SD	**Median (Min-Max)**	**Mean ±SD**	**Median (Min-Max)**	
Baseline	294.57 ±56.98	288.3 (210.0-389.0)	279.44 ±28.43	290.2 (222.8-315.1)	0.48 [A]
Week 3	276.39 ±61.07	278.0 (190.0-360.2)	271.46 ±35.40	267.3 (229.0-334.6)	0.83 [A]
Week 6	268.83 ±64.13	277.7 (183.3-357.3)	269.28 ±31.38	260.7 (230.3-330.2)	0.98 [A]
Change of mean melanin index in week 3	-18.18 ±25.59	-22.6 (-54.3-21.7)	-7.99 ±32.21	-6.5 (-65.7-34.0)	0.44[B]
Change of mean melanin index in week 6	-25.75 ±25.51	-31.7 (-60.0-15.0)	-10.17 ±31.65	6.9 (-64.4-19.0)	0.24 [B]
p-value within group					
Baseline VS week 3	0.15 [A]		1.00 [A]		
Baseline VS week 6	0.02* [A]		0.89 [A]		

A and B represented p-value from p-value from repeated measure ANOVA and Mann-Whitney U-test , * =significant at $p<0.05$

Table 4 Comparison of MASI score between and within placebo and Silymarin group.

MASI score	Placebo (n=11)		Silymarin (n=9)		p-value between group
	Mean±SD	**Median(Min-Max)**	**Mean±SD**	**Median(Min-Max)**	
Baseline	12.03 ±4.11	12.40 (6.60-17.10)	12.77 ±6.99	10.35 (4.80-26.00)	0.91 [A]
Week 3	10.12 ±3.16	10.60 (5.20-16.40)	11.00 ±5.34	8.40 (6.90-21.20)	0.65 [A]
Week 6	9.95 ±2.68	10.10 (5.10-16.50)	11.27 ±4.76	8.90 (7.10-20.00)	0.82 [A]
Change of mean MASI score in week 3	-2.17 ±2.55	-1.80 (-6.00-1.80)	-1.50 ±2.67	-0.70 (-4.80-1.80)	0.49 [A]
Change of mean MASI score in week 6	-2.34 ±3.61	-2.00 (-6.50-3.20)	-1.23 ±3.77	-0.10 (-6.10-3.20)	0.45 [A]
p-value within group					
Baseline VS week 3		0.03* [B]		0.21 [B]	
Baseline VS week 6		0.08 [B]		0.34 [B]	

A and B represented p-value from Mann-Whitney u-test and Wilcoxon Signed Ranks test

There was one participant in placebo group developed skin rash at the inner arms and withdrew from the study and three participants in Silymarin group lost to follow up. Another participant in Silymarin group felt thirsty, but only for a short period of time and can continue the treatment until the end of the study. To compare the drug compliance, in placebo group there were 52.92 tabs and 28.18 tabs left in the 3rd and the 6th week, respectively. While in Silymarin group there were 52.78 tabs and 31.11 tabs left in the 3rd and the 6th week, respectively (p-value 0.92 and 0.60, respectively). For the self-evaluation comparison, 27.3% of participant in placebo group and 33.3% in Silymarin group felt better in the 3rd week, while 54.5% of participant in placebo group and 55.6% in Silymarin group felt better in the 6th week (p-value 1.00 and 0.55, respectively). Both drug compliance and self-evaluation comparisons were no statistically significant difference between two groups. (Table 5 and 6)

Table 5 Comparison of side effects, drug compliance and self-evaluation between placebo and Silymarin group in the 3rd week.

Week3	Placebo (n=12)		Silymarin (n=12)		p-value
	Number	**Percent**	**Number**	**Percent**	
Withdraw from the study / Loss to follow up	1	8.3	3	25.0	0.59 [A]
Side effects	n=12		n=9		1.00 [A]
None	11	91.7	8	88.9	
Skin rash	1	8.3	-	-	
Feel thirsty	-	-	1	11.1	
Number of drug left (tabs)	n=11		n=9		0.92 [B]
Mean+SD	52.92±43.19		52.78±43.53		
Median (min-max)	37.5(10-160)		50(10-140)		
Self-evaluation	n=11		n=9		1.00 [A]
Same	7	63.6	5	55.6	
Worse	1	9.1	1	11.1	
Better	3	27.3	3	33.3	

A and B represented p-value from Fisher's exact test and Mann-Whitney u-test

Table 6 Comparison of side effects, drug compliance and self-evaluation between placebo and Silymarin group in the 6th week.

Week 6	Placebo (n=11)		Samarin (n=9)		p-value
	Number	**Percent**	**Number**	**Percent**	
Side effect(s)					-
None	11	100	9	100	
Number of drug left (tabs)					0.60[A]
Mean	28.18	16.624	31.11	16.351	
Median (min-max)	30(0-60)		35(10-50)		
Self-evaluation					0.55[B]
Same	3	27.3	4	44.4	
Worse	2	18.2	-	-	
Better	6	54.5	5	55.6	

A and B represented p-value from Mann-Whitney u-test and Fisher's exact test

Discussion

UV radiation is one of the significant factors in the cause of skin hepermelanosis; melasma. According to previous review literatures, Silymarin is a natural polyphenolic flavonoid, considered to be the most biologically active with potent anti-oxidant effects. It works to protect skin hypermelanosis by reduce and suppress oxidative damage from UV radiation and inhibit L-DOPA oxidation activity of Tyrosinase enzyme [9]. Furthermore, there is a study shown that topical Silymarin at concentration 14 mg./ml. had significant reduction in MASI score in melasma patients, from 16.25 +/- 2.8 to 0 in the 3rd and 4th week [10]. However, in this study found that oral intake of Silymarin 1,260 mg./day didn't show positive effect on the treatment of melasma when compared with placebo. This result corresponds with the report in 2007. Which mentioned the oral absorption of Silymarin is only about 23-47%, leading to low bioavailability of the compound [11]. In addition, other study in 1999 about tissue distribution of Silibinin in mouse by fed them with 50 mg./kg. dose of oral Silymarin and examined the maximum concentration in various tissue; the peak level of silibinin in skin tissue was 1.4 +/- 0.5 at 1 hr. after administration [12]. Therefore these two studies has supported the result that oral dose of Silymarin 1,260 mg./day might not reach the therapeutic level of melasma. However, further studies should be conducted to evaluate efficacy of oral Silymarin in melasma treatment at larger dose and longer study period.

Conclusion

Oral Silymarin 1,260 mg./day for 6 weeks has no significantly effectiveness in melasma treatment when compared with placebo. However there was no serious side effect in Silymarin group.

References

Lerner AB and Fitzpatrick TB. (1950). Biochemistry of melanin formation. Physiol Rev , 30(1) , 91-126.

Gilchrest BA, Park Hy, Eller MS, Yaar M. (1996). Mechanisms of ultraviolet light-induced pigmentation. Photochem Photobiol , 63(1) , 1-10.

Morazzoni P and Bombardelli E. (1995). Silybum marianum (Carduus marianus). Fitoterapia , 66 , 3-42.

Kren V and Walterova D. (2005). Silybin and Silymarin – new effects and applications. Biomed Pap, 149(1) , 29-41.

Choo SJ et al. (2009). Silymarin inhibits melanin synthesis in melanocyte cells. J Pharm Pharmacolo , 61 , 663-667.

Hahn G, Lehmann HD, Kurten M, Uebel H, Vogel G. (1968). Pharmacology and toxicology of silymarin, the antihepatotoxic agent of Silybum marianum(L.) Gaertn. Arzneimittelforschung , 18 , 698-704.

Mereish KA, Bunner DL, Ragland DR, Creasia DA. (1991). Protection against microcystin-LR-induced hepatotoxicity by Silymarin: biochemistry, histopathology, and lethality. Pharm Res , 8 , 273-277.

Wagner H, Diesel P, Seitz M. (1974). Chemistry and analysis of silymarin from Silybum marianum Gaertn. Arzneimittelforschung , 24 , 466-471.

Katiyar SK, Korman NJ, Mukhtar H, Agarwal R. (1997). Protective effects of silymarin against photocarcinogenesis in a mouse skin model. J Nati Cancer Inst , 89 , 556-566.

Altaei T.(2012). The treatment of melasma by Silymarin cream. BMC Dermatol, 12.

Dixit N, Baboota S, Kohli K, Ahmad S, Ali J. (2007). Silymarin: a review of pharmacological aspects and bioavailability enhancement approches. Indian J Pharmacol , 39(4) , 172-179.

Zhao J and Agarwal R. (1999). Tissue distribution of silibinin, the major active of constituent of Silymarin, in mice and its association with enhancement of phase II enzyme: implication in cancer chemoprevention. Carcinogenesis , 20(11) , 2101-2108.

Efficacy and Complications of Super-density HIFU for Full Face and Upper Neck Lift

Satit Panivhpibool[1] and Paisal Rummaneethorn[2]

Master of Science Program in Dermatology,
School of Anti-Aging and Regenerative Medicine, Mae Fah Luang University, Thailand

Abstract

High intensity focused ultrasound or HIFU is now one of the effective non-surgical procedure to treat individuals who have mild-to-moderate skin laxity. The adverse effects of HIFU are pain, transient erythema, edema, bruising and myalgia. For this research, we evaluated efficacy and safety of super-density HIFU treatment for full face and upper neck lift.

Objective: To study effect and side effect of super-density high intensity focused ultrasound to treat skin laxity for full face and upper neck lift.

Material and Methods: Eleven participants with mild to moderate skin laxity of face were performed HIFU facial lifting procedure with super-density treatment. Lifting effect evaluation was done at immediate and day 1, 3, 7, 30 and 90 after treatment by three blinded physicians and subjects themselves, using Global Aesthetic Improvement Scale (GAIS). Side effects and participants' satisfaction were estimated by questionnaires and physical examination.

Results: There was significant difference of mean GAIS since 1 day after treatment. Most participants were evaluated worse results within first 7 days; however, the GAIS substantially showed improvement since 30 days after treatment. Physician GAIS were evaluated as improved results 90.90% at day 30 and 100% at day 90. The subject GAIS showed gradual improvement since 30 days after treatment. Subjects evaluated GAIS as improved results 90.90% at both day 30 and 90. All subjects satisfied the results at day 90. The highest incidence of side effects was edema occurred in ten cases. Bruising were taken place in one case and numbness can be observed in five cases

Conclusion: HIFU facial lifting procedure with super-density treatment had the appreciable efficacy at 90 days after single treatment. Besides they were safe and well tolerated, with no significant difference of subject satisfactions and safety profiles.

Introduction

Skin laxity is one of the most concerned problems for many aesthetic patients. Laxity of the skin can cause by many factors such as genetics (chronological aging). The extrinsic factors that can cause skin laxity are gravity force, natural aging process, dermal collagen depletion, superficial fat accumulation, plastysmal band force (MacGregor, 2013)

High intensity focused ultrasound or HIFU is now a one of effective non-surgical procedure to treat individuals who have mild-to-moderate skin laxity. The demand of non-surgical procedure is now rising gradually. Many people want the treatment with less downtime, low risk profile and more natural appearance. (Pritzker & Robinson, 2014)

HIFU delivers precise focused ultrasound energy to act on specific depth of skin layers such as SMAS (Superficial musculoaponeurotic system) or parts of dermis (mid-to-deep reticular layer. After procedure, wound healing process occurs, including de novo collagen synthesis and remodeling. The adverse effects of HIFU are pain, transient erythema, edema, bruising and myalgia. Possible uncommon adverse effects are post-inflammatory hyperpigmentation, muscle weakness, transient numbness, and striated linear skin patterns or wheals (Fabi et al., 2015)

Oni et al, 2014 showed the effectiveness of Ulthera for improving skin laxity and tightening in the lower face with reporting improvement by two-thirds of patients and nearly 60% of blinded reviewers at day 90. However, Lee et al, 2012 displayed multiple pass technique for tightening of skin laxity of lower face and neck with effective results. For this research, the researcher made an effort to evaluate efficacy and complications of super-density treatment.

Objectives

To study effect and side effect of super-density high intensity focused ultrasound to treat skin laxity for full face and upper neck lift.

Material and Methods

Eleven participants with mild to moderate skin laxity of lower face were enrolled in the study. 7 men and 4 women were performed HIFU facial lifting procedure with super-density treatment. All enrolled

subjects had been taken a photograph of the straight face by digital camera in each follow-up visit. Clinical visits were scheduled at immediate after treatment and after 3, 7, 30 and 90 days. These representative clinical photographs were evaluated by three physicians blinded to treatment group and timing of the photographs, using Global Aesthetic Improvement Scale (GAIS) (1 = Worse, 2 = no change, 3 = improved, 4 = much improved, 5 = very much improved), and every follow-up visit, the researcher assigned all subjects to estimate the effect of facial lifting after treatment by themselves. Comparing the photograph of the straight face was done by subjects and also rated by using GAIS. The researcher carefully observed about side effects after treatment by taking the history and physical examination, and then recorded the noticeably founded incidences.

Statistics for Data Analysis

Statistical analysis was performed by using SPSS version 22 (IBM, Chicago, IL, USA). Comparisons between treatment result at each visit (day 0, 1, 3, 7, 30 and 90) and baseline were tested by using Wilcoxon Signed-Ranked test. Statistical significance was determined at p-value < 0.05.

Results

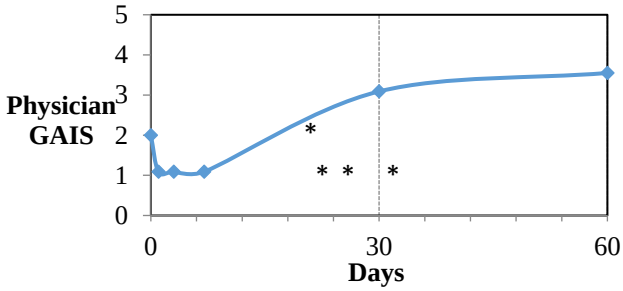
The collected data of evaluated GAIS by 3 physicians were calculated including statistically comparing results between HIFU facial lifting with super-density treatment and baseline in each visit. There were significant differences of mean GAIS since 1 day after treatment. (p = 0.002) Within first 7 days after treatment, most participants were evaluated worse with GAIS 1.09; however, the GAIS substantially showed improvement since 30 days after treatment. Physician GAIS were evaluated as improved results 90.90% (10/11) at day 30 and 100% (11/11) at day 90.The mean GAIS were illustrated in linear graph pattern as shown in the picture 1.

Significant differences of mean subject GAIS were noticeable since immediate after treatment (p = 0.046). Most subjects estimated worse results (GAIS 1.09), and the GAIS showed gradual improvement since 30 days after treatment. Subjects evaluated GAIS as improved results 90.90% (10/11) at both day 30 and 90.The mean GAIS were illustrated in linear graph as shown in the picture 2

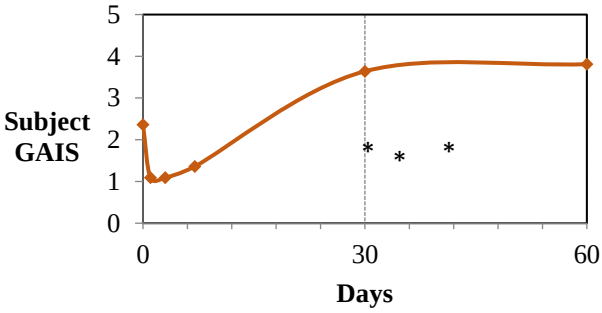
After being treated with HIFU facial lifting with super-density treatment, the subjects rated their satisfaction in each follow-up visit. Afterwards, the researcher conducted to find out statistical tendency of subject satisfaction scores changes as shown in the

picture 3.

The highest incidence of side effects was edema occurred in ten cases (10/11= 90.90%). Bruising were taken place in one case (1/11 = 9.09%) and numbness can be observed in five cases (5/11 = 45.45%). Edema and numbness could remark at day 7 after treatment, meanwhile bruising could found immediately after treatment. However, other side effects did not exist in this study.



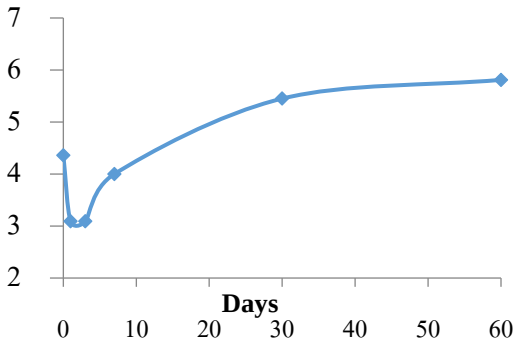
Picture 1 Linear graph shows comparison of mean global aesthetic improvement scale in each visit evaluated by physicians



Picture 2 Linear graph shows comparison of mean global aesthetic improvement scale in

Patient Satisfaction Score

each visit evaluated by subjects



Picture 3 Linear graph shows comparison of mean changes of subject satisfaction scores in each period

Discussion

The results of this study confirmed that HIFU facial lifting with super-density treatment was safe and effective treatment for full face and neck. The facial lifting effects were seen in 10 subjects (90.90%) at day 30 and all subjects (100%) at day 90. According to the previous study, Lee et al (2012) reported clinical improvement of facial lifting at day 90 (80%) after treatment and subjective improvement (90%) by patient self-assessment. For such difference, it might be caused by investigators' variation in the ratings of individuals, so the results of this study seem to be quicker onset of lifting; however, this imply that super-density treatment may possibly lead to earlier result.

This study showed the similarity of evaluated physician GAIS and subject GAIS that declined in first 7 days, and gradually increased since day 30 with reaching the statistical significance compared to baseline at any time point. This consistent result suggested that the participants might have experienced worse results within 7 days after treatment, but the facial lifting effect could be certainly remark after treatment at least 30 days.

For the subject's assessment of satisfaction scores, all participants dissatisfied the results in first 7 days, but obviously higher satisfaction scores were exhibited at day 30 and 90. It is possible that dissatisfaction might be associated with adverse events after treatment especially edema and higher satisfaction scores was evaluated when these complications subsided with observable lifting effects. Even though the outcomes were subjective, it exhibited relationship with mean physician GAIS and subject GAIS in the same way. This implied such association shows the lifting effect existed, the more subject satisfaction became.

In terms of safety, treatment-related adverse events that found in this study included temporary edema on treatment sites in 10 participants (90.90%) at day 1. Bruising was found immediately after treatment in one case (9.09%). 5 participants (45.45%) experienced symptom of transient numbness on the treatment site since day 1 and gradually spontaneously recover within weeks. Besides, all subjects had only a minimal pain level during treatment. It is of note that no event of other side effects has been reported, This also suggested about safety of super-density treatment. However, almost all subjects (90.90%) had gotten temporary edema as the most common treatment-related side effect in this study.

Conclusion

The results from this study demonstrate that HIFU facial lifting procedure with super-density treatment had the appreciable efficacy at 90 days after single treatment. Besides they were safe and well tolerated, with no significant difference of subject satisfactions and safety profiles.

Suggestions

The study may be tested to compare between super-density treatment and standard treatment for determination of optimal dosage used for treatment of facial laxity. Besides, the cosmetic use of super-density treatment can be advantageous to tightening and lifting lax skin in other anatomical regions (e.g. décollatage, neck, upper arms, thighs, knees), so this can be studied the efficacy and safety for various applications.

References

Fabi SG1, Goldman MP.(2014). Retrospective evaluation of micro-focused ultrasound for lifting and tightening the face and neck. *Dermatol Surg.* 40, 569-575.

Lee HS, Jang WS, Cha YJ, Choi YH, Tak Y, Hwang E, Kim BJ, Kim MN. (2012). Multiple pass ultrasound tightening of skin laxity of the lower face and neck. *Dermatol Surg.* 38, 20-27.

MacGregor JL1, Tanzi EL. (2013). Microfocused ultrasound for skin tightening. *Semin Cutan Med Surg.* 32,18-25.

Pritzker RN1, Robinson DM2. (2014) Updates in noninvasive and minimally invasive skin tightening. *Semin Cutan Med Surg.* 33,182-187.

Woolery-Lloyd H1, Kammer JN. (2011). Skin tightening.*Curr Probl Dermatol*. ;42:147-52.

Factors Related to Consumer's Decision in Purchasing Herbal Supplements in Bangkok, Thailand

Monthicha Rakcharoen[1] and Dr. Gloria S. Chavez[2]

[1] A student of Master's of Business Administration,
[2] Senior lecturer, Graduate school of Business, Assumption University in Thailand

Abstract

The herbal supplements market has grown in the recent years. Several kinds of herbal supplements are available in the market. The purpose of this research is to identify the key factors that are related with the decision to purchase herbal supplements in Bangkok, Thailand in order for the herbal supplements suppliers to enhance opportunity to sell the products. A survey questionnaire was used to gather data from a sample of 385 the target population who purchased herbal supplements. Correlation Coefficient was applied to determine the relationship between health consciousness, product knowledge, price consciousness, sales promotion and purchase decision toward herbal supplements. The findings of this research show that all factors are significant. Health consciousness and product knowledge have a strong positive relationship with purchase decision toward herbal supplements. Also, price consciousness and sales promotion showed a weak positive relationship with decision to purchase herbal supplements. Furthermore, the researcher provides several recommendations for the herbal supplements suppliers to develop strategies for the product offering.

Keywords – Health consciousness, product knowledge, price consciousness, sales promotion, and purchase decision.

Introduction

Currently, people have become more concerned about being healthy and good looking. Health conscious people will make it a point to use several alternatives to be healthy both internally and externally by going through exercises, annual physical examinations, having themselves vaccinated and taking supplements. This trend has influenced consumer behavior. Herbal supplements are a new alternative for people who are health and beauty conscious. People consume herbal supplements for many purposes, some for prophylactic purposes, some for treatment and some for beauty. The herbal supplements market has grown in recent years because most people tend to choose natural products or product made from herbs, to respond to the belief that consuming herbs can promote healthy skin and body. There are several brands of herbal supplements available in the market including Thai and international brands, which have been launched using intensive marketing. Therefore, the researcher would like to study the factors related to the decision to purchase herbal supplements which is very important for them to be able to survive in the market.

Literature Review

Health Consciousness

According to Hill and Lynchehaun (2002), health consciousness is the consumers' understanding of changing health status and health requirements. Also, health conscious consumers care about the desired degree of well-being and they will put effort on having good health (Newsom et al., 2005). People who are health conscious will make it a point to use several alternatives for having a good health and also taking healthy actions such as consuming supplements product (Ling-Yu and Shang-Hui, 2013).

Product Knowledge

Different levels of product knowledge by consumers vary in consumer's perception about product attributes (Laroche, Bergeron, & Goutaland, 2003). Product knowledge of customers is based on how much information about the product is perceived and how known information are recollected and used for purchase decision (Chuang and Tsai, 2005). Consumers, who have higher levels of product knowledge, have complicated decision making process and tend to have less appraisal bias than consumers with lower levels of product knowledge. Due to the fact that most consumers do not clearly understand the purpose for consuming supplements, knowledge factors are a main impact of consumer trust and behavioral intention (Hughner, McDonagh, Prothero, Shultz, & Stanton 2007). According to Gifford and Bernard (2006) and Yiridoe, Bonti-Ankomah, and Martin (2005), knowledge of products affects purchasing decision of that product. Better knowledge of product tends to be related with higher behavioral intention (Vermeir and Verbeke, 2006). Furthermore, Gracia and Magistris (2008) revealed that product knowledge can enhance attitude

and likelihood to purchase and also promote consumption among current consumers. Product knowledge is perceived to be a crucial antecedent of customer trust (O'Fallon, Gursoy, & Swanger 2007). In addition, Demeritt (2002) claimed that inadequate knowledge of product is considered as critical barriers to purchase products.

Price Consciousness

According to Jin and Sternquist (2004), price conscious consumers consider price as the resource they need to pay attention to when they purchase a product thus, they will put more effort such as going to several stores to get the best price. In purchasing the selected product, low price is more important for price conscious consumers (Kukar-Kinney et al., 2012). Furthermore, Martínez and Montaner (2006) revealed that purchasing discounted items provide more savings for price conscious consumers. Also, the more price conscious the consumer is, the more likely the consumer will appeal to sales promotions. Moreover, price conscious consumers are likely to place more importance to the quality of product and they are brand switchers to lower priced products.

Sales Promotion

Sales promotion is a marketing communication tool which includes special offers or incentives that encourage customers to buy a product (Alvarez and Casielles, 2005). De Run et al. (2010) stated that sales promotion is applied to influence current customers to increase their purchasing and encourage new customers to buy the product. It also includes communication activities to stimulate immediate sales. According to Ndubisi and Chiew (2006), sales promotion becomes more important for manufacturers and retailers around the world since it a most appropriate tool to encourage customers to try products or services and help trader to achieve their goal. There are several types of sales promotion technique used widely such as sampling, coupon, premium, point collection among others. (De Run, Jee, & Nathawut, 2010). Furthermore, Kotler and Keller (2012) revealed that sales promotion are short term incentives to provoke purchase of product or service. Also, it includes a wide variety of promotion tools and activities which are designed to create interest such as short term marketing events apart from advertising, direct marketing, personal selling and public relation to stimulate market response and attract buyers

Purchase Decision

Kotler and Keller (2012) revealed that understanding consumer purchasing decision becomes an important factor that has direct influence on the business's performance and also, studying consumer decision making process is a crucial marketing part to be able to compete in the market. Studying how people select, purchase, consume and dispose the product and factors such as price, past experience and product attribute in which consumers base their purchase decisions is very important. According to Kotler et at. (2013), when the consumer makes a purchase decision, it is a primary stimulation and response to external stimuli such as marketing tools which will impact consumer buying decisions. Abendroth (2011) studied the factors influencing purchase opportunity and purchase likelihood of the product and also, the stimulus for people to make initial purchase decision. The study applied the specific scenario to stimulate purchase, which is either to "purchase the product" or "not purchase the product". Moreover, the study discovered more intensely the decision making process. A consumer buys a selected product during the evaluation stage and the purchase decision follows the evaluation. What is more, the consumer always makes different purchase decisions (Clow and Baack, 2002). There are various factors that play an important role in making the decision to purchase the product (Ares, Gimenez, & Gambaro, 2008). Also, purchase decision is perceived as the process wherein consumers evaluate alternative products based on various factors.

Research Framework and Methodology

The conceptual framework of this study was developed based on four research models. The first research model was proposed by Konuk (2015) whose studied was about "The effects of price consciousness and sale proneness on purchase intention towards expiration date-based priced perishable foods". The second research model based on a study conducted by Hsu, Chang, & Lin, (2016) who investigated about "An analysis of purchase intentions toward organic food on health consciousness and food safety with/under structural equation modeling". The third research model was developed by Teng and Wang (2015) whose study was about "Decisional factors driving organic food consumption: Generation of consumer purchase intentions". The last research model was developed by Weng and Run (2013) whose study was about "Consumers' personal values and sales promotion preferences effect on behavioural intention and purchase satisfaction for consumer product".

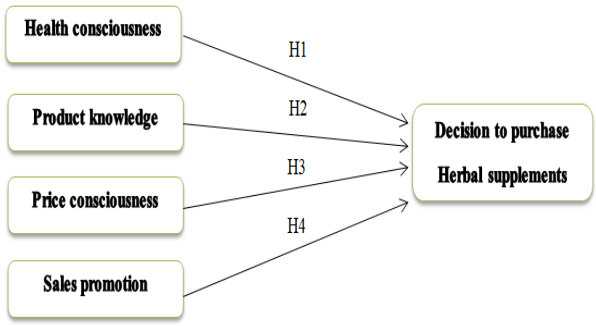


Figure 1: The modified conceptual framework of the "Factors Related to Purchase Decision toward Herbal Supplements in Bangkok, Thailand"
Source: Developed by the researcher for this study

H1: There is a significant relationship between health consciousness and purchase decision toward herbal supplements.
H2: There is a significant relationship between product knowledge and purchase decision toward herbal supplements.
H3: There is a significant relationship between price consciousness and purchase decision toward herbal supplements.
H4: There is a significant relationship between sales promotion and purchase decision toward herbal supplements.

Research Methodology

This research used a descriptive and correlational research design. Descriptive research is used to determine and describe the characteristics of a group of population (Shields and Rangarjan, 2013). According to Mcleod (2008), a correlational study is a quantitative method of research in which there are two or more variables and the objective is to determine if there is a relationship between these variables. The study also used a six point Likert scale survey questionnaire to collect data from consumers who have purchased herbal supplements in Bangkok, Thailand. Zikmund (2010) stated that population refers to a group of people who share a common set of characteristics and similar interest. The target population of this study are those who have purchased herbal supplements and the respondents of this research are people whose ages are between 18 years old to 70 years old and live in Bangkok, Thailand. The researcher used non-probability sampling method particularly convenience sampling and snowball sampling techniques.

Stage 1: Pilot test to check the reliability of the questionnaire by distributing the questionnaire to 30 respondents who have experienced purchase of health supplements in Bangkok, Thailand.

Stage 2: Convenience and snowball sampling

The researcher collected the data by using convenience sampling technique from people who have purchased herbal supplement, who are convenient and willing to answer the research's questions. Snowball sampling was also used which involves collecting data from respondents who have been referred by other respondents (Neuman, 2007). The 385 questionnaires were collected from February to March 2016 at Sangsuriya (2002) Co., Ltd and herb shops in Bangkok area.

The researcher developed a self-administered questionnaire to collect the primary data with the purpose of determining the key factors related to purchase decision toward herbal supplements. The questionnaire was designed with three parts. The first part is the screening questions which are about the target population who have purchased herbal supplements in Bangkok. The second part focused on the dependent and independent variables which are those related factors associated with consumers' purchase decision toward herbal supplements with a total of 25 questions. The researcher applied the 6-point Likert Scale for this part as respondent's attitude indicator or level of perception by selecting how strongly agree or disagree with each question (Zikmund, 2010). The last part involves the demographic factors or personal information. The demographic data include gender, age, marital status, education level, and monthly income which describe the population characteristics of consumers. Field (2010) suggested that Cronbach's Alpha that measure the reliability of the instruments should be equal or above 0.7 to be an acceptable value. Table 1 shows the Alpha tests of all variables. The total variables are equal to 0.940, which is greater than 0.7.

Table1: Reliability Test Result

Variables	**Alpha Test (α-Test)**
Health Consciousness	.972
Product Knowledge	.871
Price Consciousness	.871
Sales Promotion	.868
Purchase Decision	.912
All variables	**.940**

Result and Discussion

Results

The researchers utilized a Statistical program to analyze the data that was collected from 385 respondents. Descriptive Analysis and Pearson Correlation were used as data analysis techniques to analyze the related factors.

Descriptive analysis

Table 2: Demographic Profile of Respondents

Variables	***f***	**%**
Gender		
Male	121	31.4
Female	264	68.6
Total	385	100
Age		
Less than 20 years old	18	4.6
20-35 years old	184	47.8
36-50 years old	130	33.8
More than 50 years old	53	13.8
Total	385	100
Marital Status		
Single	179	46.6
Married	178	46.2
Divorced	24	6.2
Others	4	1
Total	385	100
Education Level		
Below Bachelor Degree	147	38.2
Bachelor Degree	194	50.4
Master Degree	44	11.4
Doctoral Degree	0	0
Total	385	100
Monthly Income		
Below 20,000 Baht	159	41.3
20,000-30,000 Baht	97	25.2
30,001-40,000 Baht	66	17.1
More than 40,000 Baht	63	16.4
Total	385	100
Total	**385**	**100**

Inferential Analysis

The researcher tested the hypotheses of the research model by applying the Pearson Product Moment Correlation Coefficient to examine the relationship between each variable.

Table3: Hypothesis Testing Result

Hypothesis	**p value**	**Coefficients (r)**	**Level of Correlation**	**Results**
H1	.000	.770	Strong relationship	Accepted
H2	.000	.699	Strong relationship	Accepted
H3	.000	.259	Weak relationship	Accepted
H4	.000	.221	Weak relationship	Accepted

As indicated in Table 5.3.1, the results from the Pearson Correlation showed that the overall p value is equal to .000, which is less than .01 (.000 < .01). It means that all of the null hypothesis was rejected. Thus, there is a significant relationship between all independent variables which are product knowledge, health consciousness, price consciousness, sales promotion and the dependent variable which is purchase decision toward herbal supplements.

The first hypothesis on testing the relationship between health consciousness and decision to purchase herbal supplements shows a Coefficient (r) of .770 , this means that there is a strong positive relationship between health consciousness and purchase decision.

The second hypothesis on testing the relationship between product knowledge and decision to purchase herbal supplements shows that the Coefficient (r) is equal to .699. This implies that there is a strong positive relationship between product knowledge and purchase decision.

The third hypothesis on testing the relationship between price consciousness and decision to purchase herbal supplements shows that the Coefficient (r) is equal to .259, this means that there is a weak positive relationship between price consciousness and purchase decision.

The last hypothesis is about testing the relationship between sales promotion and decision to purchase herbal supplements. The result shows that the Coefficient (r) is equal to .221, this means that there is a weak positive relationship between sales promotion and purchase decision.

Discussion

The objective of this study was to determine the factors such as health consciousness, product knowledge, price consciousness, and product knowledge that are related with consumer decision in purchasing herbal supplements in Bangkok, Thailand. Furthermore, Food and Drug Administration of Thailand restricts advertising on herbal supplements to

protect consumers. Thus, information and benefits of herbal supplements provided are limited. Also, the ingredients of herbal supplements are controlled by this agency.

The results of the study show that health consciousness, product knowledge, price consciousness, and sales promotion have a significant relationship with the purchase decision of herbal supplements. All the hypotheses were accepted with a p value equal to .000. All the independent variables have a significant relationship with the dependent variable.

The findings of this study are consistent with the result of previous empirical studies. The study of Konuk F. (2015) showed that price consciousness affect purchase intention. In addition, Hsu Y.,et.al. (2016) revealed that health consciousness has a significantly positive impact on purchase intentions. It also indicates that subjective knowledge and health consciousness are important factors affecting purchase decision. Moreover, Santini et al. (2015) indicated that sales promotions affect consumer purchase intention. Also, Teng and Wang (2015) pointed out that trust significantly mediates the relationships between revealing information, perceived knowledge, and organic purchase intentions. There should be emphasis on how to use product knowledge to encourage consumers to buy product as an effective marketing strategy.

Limitations, Conclusion and Recommendation

Limitations

This research investigated the factors related to consumer's decision in purchasing herbal supplements in Bangkok, Thailand. Due to the limitations of time, Feb-March 2016, the data cannot be collected from all herbal supplements consumers in Bangkok, Thailand. Moreover, this research is not able to study all of the people who have experience in purchasing herbal supplements around the world. In addition, only the variables of product knowledge, price consciousness, health consciousness, sales promotion are used in this research to determine the consumers' decision to purchase herbal supplements.

Conclusion

The results of the study show that health consciousness and product knowledge have a strong relationship with purchase decision toward herbal supplements. On the other hand, price consciousness and sales promotion have a weak relationship with purchase decision toward herbal supplements. Therefore, herbal supplements suppliers should focus on these key factors which are health consciousness, product knowledge, price consciousness, and sales promotion in order to develop marketing strategies for their product offerings.

Recommendations

Based on the findings of this study, all the factors: health consciousness, product knowledge, price consciousness, and sales promotion have a relationship with purchase decision of herbal supplements. Moreover, health consciousness and product knowledge have a strong relationship with decision to purchase herbal supplements. The researcher recommends that health consciousness and product knowledge should be the focus of herbal supplement suppliers. They should target health conscious consumers as their market and concentrate on this group of people who are most likely to buy herbal supplements. Also, health conscious consumers group are growing, the suppliers should develop marketing strategies and communicate to this target customer and tell them that taking herbal supplements is necessary to maintain a good health and there is no residue to encourage them to buy the products. In terms of product knowledge, the proper information about the herbal supplements should be provided to consumers such as why they need to take herbal supplements and what benefits herbal supplements can provide.

Consumers should be informed that they can take herbal supplements for several purposes for example, to protect oneself from potential health threats as well as beauty benefits. Consumers should be provided with ample information that different types of herbal supplements serve different purposes.

Further, the suppliers should include in their communication that herbal supplements are suitable for people of all ages and all genders. Moreover, the researcher also suggests that price and sales promotion should also be attended to since they are related with consumers' purchase decision of herbal supplements. Price conscious consumers should be given information that it is worth to pay the price for herbal supplements as it contributes to their health. Herbal supplements price is perceived to be expensive so the suppliers should explain the price with reasonable support. The suppliers should also explain consumers why it is more expensive than other kinds of supplements or how it is superior or better than others.

Finally, sales promotion may be offered to motivate consumers to buy herbal supplements because some consumers will buy herbal supplements only when it is offered on sales promotion. For example, offering price discounts to encourage consumers to try the product or offering trial size of herbal supplements to let consumer experience the product. Also, some consumers tend to buy more herbal supplements when there is a sales promotion offer. Sales promotion, such as price discounts or buy one get one free, may be used to boost sales volume.

References

Abendroth, L.J. (2011).The souvenir purchase decision: effects of online availability. International

Journal of Culture, Tourism and Hospitality Research, 5(2), 173 – 183

Alvarez, A.B. & Casielles, R.V. (2005). Consumer evaluations of sales promotion: the effect on brand choice. European Journal of Marketing, 39, 54-70.

Ares, G.,Gimenez, A. & Gambaro, A. (2008). Sensory shelf life estimation of minimally processed lettuce considering two stages of consumers decision-making process. Appetite, 50, 529-535.

Chuang, S., & Tsai, C. (2005). The impact of consumer product knowledge on the effect of terminology in advertising. Journal of American Academy of Business, 7(1), 223-227.

Clow, K. E. & Baack, D.(2002). Integrated advertising, Promotion and Marketing Communications. Journal of Marketing Communications, 9(3),193-194

De Run, E.C., Jee, T.W. & Nathawut, S. (2010),Thailand, Asia Business Research Corporation Limited, Wellington.

Demeritt, L. (2002). All Things Organic 2002: A Look at the Organic Consumer, The Hartman Group, Bellevue, WA.

Field, A. (2009). Discovering Statistics using SPSS. Sage: London.

Gifford, K. & Bernard, J.C. (2006). Influencing consumer purchase likelihood of organic food.International Journal of Consumer Studies, 30(2),155-163.

Gracia, A. & Magistris, T.D. (2008). The demand for organic foods in the south of Italy: a discrete choice model.Food Policy,33(5), 386-396.

Hill, H. & Lynchehaun, F. (2002). Organic milk: attitudes and consumption patterns. British Food Journal, 104(7), 526 – 542.

Hsu,S.Y., Chang, C.C.& Lin, T.T. (2016).An analysis of purchase intentions toward organic food on health consciousness and food safety with/under structural equation modeling.British Food Journal,118 (1), 200 – 216

Hughner, R.S., McDonagh, P., Prothero, A., Shultz, C.J. II &Stanton, J. (2007). Who are organic food consumers? A complication and review of why people purchase organic food. Journal of Consumer Behaviour, 6 (2-3), 94-110.

Jin, B. & Sternquist, B. (2004). Shopping is truly a joy.The Service Industries Journal, 24(6),1-18.

Konuk, F.A. (2015).The effects of price consciousness and sale proneness on purchase intention towards expiration date-based priced perishable foods. British Food Journal, 117(2), 793-804.

Kotler, P., Keller, K.L., Koshy, A. & Jha, M. (2013). Marketing Management: South Asian Perspective', Pearson, 14th edition.28-40.

Kukar-Kinney, M., Rigway, N.M. & Monroe, K.B. (2012). The role of price in the behavior and purchase decisions of compulsive buyers. Journal of Retailing, 88(1), 63-71.

Laroche, M., Bergeron, J. & Goutaland, C. (2003). How Intangibility Affects Perceive Risk: the Moderating Role of Knowledge and Involvement. Journal of Services Marketing, 17(2), 122-140.

Ling-Yu, M. W. & Shang-Hui, L. (2013). A Study On The Relationship Amidst Health Consciousness, Ecological Affect, And Purchase Intention Of Green Production. International Journal Of Organizational Innovation, 5, 124-137.

Martínez, E. & Montaner, T. (2006).The effect of consumer's psychographic variables upon deal-proneness. Journal of Retailing and Consumer Services, 13(3),157-168.

McLeod, S. A. (2008). Qualitative Quantitative. Retrieved from www.simplypsychology.org/qualitative-quantitative.html

Ndubisi, N.O. & Chiew, T.M. (2006). Awareness and usage of promotional tools by Malaysian consumers: the case of low involvement products. Management Research News,29, 28-40.

Kotler, P., & Keller, K. L. (2012). Marketing management(14th edition). Upper Saddle River, N.J: Pearson Prentice Hall

Neuman, W.L. (2007). Basics of Social Research: Qualitative and Quantitative Approaches, 2/E, Pearson Education. Whitewater, Wisconsin.

Newsom, J., McFarland, B., Kaplan, M., Huguet, N. &Zani, B. (2005).The health consciousness myth: implications of the near independence of major health behaviors in the North American population. Social Science & Medicine, 60 (2), 433-7.

O'Fallon, M.J., Gursoy, D. & Swanger, N. (2007).To buy or not to buy: impact of labelling on purchasing intentions of genetically modified foods. International Journal of Hospitality Management,26(1),117-130.

Santini, F.D.O., Ladeira, W.J., Sampaio, C.H., & Falcão, C.A. (2015). Perception of value, attractiveness and purchase intention: revisiting sales promotion techniques. Review of Business Management, 17(57), 1173-1192.

Shields, P.M. & Rangarajan, N. (2013). A Playbook for Research Methods: Integrating Conceptual frameworks and Project Management. Stillwater, OK: New Forums Press

Teng, C.C. & Wang, Y.M. (2015). Decisional factors driving organic food consumption. British Food Journal,117(3),1066 – 1081

Vermeir, I. & Verbeke, W. (2006). Sustainable food consumption: exploring the consumer 'attitude-behavioural intention' gap.Journal of Agricultural and Environmental Ethics,19(2), 169-194.

Weng,J.T. & Run, E.C. (2013). Consumers' personal values and sales promotion preferences effect on behavioural intention and purchase satisfaction for consumer product. Asia Pacific Journal of Marketing and Logistics, 25(1), 70 – 101

Yiridoe, E.K., Bonti-Ankomah, S. &Martin,R.C. (2005). Comparison of consumer perceptions and preference toward organic versus conventionally produced foods. a review and update of the literature. Renewable Agriculture and Food Systems, 20(4), 193-205.

Zikmund, W.G. (2010). Business Research Methods,8th Edition. Oklahoma State University. South-Western Publishing Company.

.

Grief on Patient's Death among Filipino Nurses

Juniel B. Tagarino, RN, MAN, AEMT

Clinical Instructor, Samar State University – College of Nursing and Health Sciences, Catbalogan City, Samar, Philippines, 6700
Jbtagarino1987@gmail.com

Abstract

Grief of nurses following patient's death may influence their response to work that brings impact into their professional roles. Caring for dying patient and his/ her family is often describe by nurses as the most painful and stressful elements of the nurses role, thus thinking and attitude changes by reviewing, ranking values, becoming wiser shedding illusions about the immortality, viewing the world more realistically and re-evaluating religious or spiritual beliefs. This study determined nurse's grief following patient's death which identifies the changes experience by nurse's after patient's death and their ability to return functioning following patients death. The researcher employed a descriptive correlational research design to determine nurse's grief following patient's death. A standardized instrument was utilized in this study. A total of 137 Nurses in different area of the hospital were taken as respondents. Statistical tool used in this study such as frequency count, percentage, mean, standard deviation and Pearson product moment correlation in analysing the raw data collected. This study revealed that the number of patient's death experienced by the respondents is influenced by the changes experienced as a result of patient's death. Age and religion are significant to nurse's ability to return functioning following patients death. In conclusion, it is observed that majority of the Filipino Nurses experience small degree of changes after having experience patient's death thus a high chance to return to normal functioning but feel a minor physical symptoms, but age and years of working experience plays an important contribution to the nurse's ability to cope with the experience on patient's death. Base on the findings of the study it is recommended that a nurses grief management program should be established to support heath care workers in the grieving process and participation from nurses is important.

Keywords: Grief, Filipino Nurses, Death

Introduction

All people grieve when they experience life changes and losses. Grief is the process and emotions that we experience when our important relationship are significantly interrupted or more frequently ended, either through death, divorce, relocation, destruction or some similar process. (Dombeck,2006) Grieving often causes a person to change beliefs about self and the worlds benevolence, the meaning of life as related to justice, and a sense of destiny or life path. (Zisook and Zisook,2005)People may suppress emotional response to the loss or become obsessively preoccupied with the deceased person or lost object. Others actually may suffer from clinical depression when they cannot make progress in the grief process. (Zhang, El-Jawahri, and Prigerson,2006)

Physiologic symptoms and problems associated with grief responses are often a source of anxiety and concern for the grieving person as well as for friends or caregivers (Zisook and Zisook,2005) Nurses attribute the lack of support and training on end-of-life care, there is a gap on the knowledge and attitudes of critical care nurses, thus such lack of knowledge is often reflected in the care of these nurses provide to their dying patients. (Salahuddin,2008) Death in the intensive care environment can seem unnatural and difficult to deal with for nurses. It is thought to be very painful and stressful process for the nurses who provides continuous care to the patients. (Beckstrand and Kirchhoff,2005) Technology has become a barrier to nurses' caring ideals, nurses find themselves caring for a piece of technology rather than the patient himself (Beckstrand and Kirchhoff,2005) thus nurses must find a way to care for the dying patient, to make the death a satisfying and peaceful one. (Tomey and Alligood, 2006)

Stress levels are statistically higher in hospital based nurses who have frequent exposure to dying patients than in those who are not exposed (Mitchell, Sakraida, DysartGale and Gadmer, 2006) this stress maybe due in part to a nurse's lack of confidence in providing appropriate care to the dying (Brajtman,2007) but there is little research available on the coping mechanisms nurses use to cope with particular stressor and some of the nurses simply focus on other tasks to distract them from emotional toll of the dying patient (Hopkinson,2005) thus if this stress is not appropriately dealt with, it will continue to cause anxiety when caring for the dying patient.

Age	Male	Female	Total	WM
30	0	3	3	2.19
29	0	6	6	4.38
28	5	4	9	6.57
27	4	3	7	5.11
26	0	4	4	2.92
25	0	5	5	3.65
24	25	39	64	46.72
23	10	14	24	17.52
22	7	4	11	8.03
21	0	4	4	2.92
Total	51	86		
Percentage	37.23	62.77		
Average	24.16	24.60		24.44
SD	1.74	2.18	2.02	

Objectives

This study determined the grief on patient death among Filipino nurses. This will also determine their coping mechanism towards the grieving process.

Research Methodology

Design: This study utilized descriptive correlational type of research to determine the grief on the patient's death among Filipino nurses.

Participants: This study was conducted in hospitals in Samar, Philippines. There were around 137 nurses who qualified to be the responder of the study. The criterion in selection of the responders were: (a) have experience the death of more than one patient, (b) male or female, (c) signed a contract in the hospital that they are affiliated with as staff nurses, (d) have more than one year experience in the hospital field, and (e) agreed and signed consent to participate in the study.

Ethical consideration: An approval letters to the Ethics Committee of the provincial health office and hospitals prior to the conduct of the investigation and was approved. The Confidentiality of information and anonymity of the respondents were maintained by using only code number instead the name of the respondents.

Instrumentation: A standardized instrument was used to gather the data on the grief on patient's death among Filipino nurses. The questionnaire by Dr. Corrine M.

Walijarvi was adopted who studied the relationship between the six therapeutic elements and progress in the grief journey. The researcher makes modification in gathering the necessary data to answer the research questions in which there were four parts of the questionnaire that contains forty-two items.

Main results and discussion:

Majority of the nurse-respondents are female 86 out of 137. Female respondents turned out to be older than male respondents in as much as their means turned out to 24.48 years old and 23.51 years correspondingly. Majority of the respondents is single that is, 103 out of 137 or 75.18%.The highest employment status is full time with a total of 87 or 63.50%.Majority of the respondents are Roman Catholic with frequency of 119 or 86.86%. The highest number of years in the hospital field is 1 year with a total 78 or 56.93%. The highest number of patient's death experienced by nurse-respondents belonged to 6 and up patient's death with frequency of 98 or 71.53.

Table 1.1 Age and sex distribution of the nurse respondents

Reflected in table 1.1 is the information pertaining the gender and age distribution of the nurse respondent's. It reflects that majority of the respondents are 24 years old or 46.72% form the total population. In general the age distribution of the nurse-respondent clustered around 24.44 with a standard deviation of 2.02. The female respondents turned out to be older than male respondents in as much as their mean turned out to be 24.60 years and 24.16 years correspondingly.

Table 1.2. Profile of the Nurse – respondents in terms of Civil Status

Civil Status	F	Percentage
single	103	75.18
married	24	17.52
widow	0	0
other	10	7.30
Total	137	100

Table 1.2 reflects that 103 or 75.18% of the nurse respondents are single. As a whole this denotes that the nurse-respondents are dominated with single status. This denotes that the nurse respondents are more likely to share their feelings of grieving to patient's death with their family, friends or co-workers.

Table 1.3 Employment Status

Status	F	Percentage
Fulltime	87	63.50
Part time	8	5.84
Volunteer	12	8.76
Job order	30	21.90
Total	137	100

Table 1.3 reflects the employment status of the nurse-respondents. It shows that the highest employment status is full time with a total of 87 or 63.50%. as a whole the hospitals are staffed with fulltime nurses which denotes that the nurse-respondents worked long period of time and had a longer working relationship with patients.

Table 1.4 Religion

Status	F	Percentage
Roman Catholic	119	86.86
Born again	9	6.57
Protestant	3	2.19
latter day	4	2.92
others	2	1.46
Total	137	100

Table 1.4 shows the religion of the nurse-respondents. It reveals that majority of the nurse-respondents are roman catholic with a total of 119 or 86.86%.as a whole , the nurse-respondents have spiritual belief denotes that they can rapidly resolve their grief to their patients who died.

Table 1.5. Years of employment

Years of employment	F	Percentage
1	78	56.93
2	26	18.98
3	10	7.30
4	6	4.38
5	9	6.57
6 and Up	8	5.84
Total	137	100
Average	2.02	

Table 1.5 shows the years of employment of nurse-respondents. It shows that most of them are employed for 1 year with total of 78 or 56.93%

Table 1.6. Number of death experience among Nurse -respondents

Number of deaths	F	Percentage
1	18	13.14
2	12	8.76
3	9	6.57
4	0	0.00
5	0	0.00
6 and Up	98	71.53
Total	137	100
Average	4.8	

Table 1.6 shows the number of death experience or witnessed by Nurse –respondents. this reflects that most of them witnessed 6 or more patients death with a total of 98 or 71.53%

Table 2. Changes of nurse-respondent's as a result of the death of the patient

Statement	4	3	2	1	0	Mean	Interpretation
1. I have difficulty with personal relationships or have trouble sleeping or eating properly.	0	10	30	41	56	0.96	NC
2. I feel I am not a good nurse because I lost my patient.	0	19	10	64	44	1.04	NC
3. I feel reluctant to get close to other patients.	0	8	17	36	76	0.70	NC
4. I feel angry and upset after seeing my patient.	0	10	28	41	58	0.95	NC
5. I changed my priorities about what is important in life.	13	17	18	18	71	1.17	NC
6. I developed new interests.	14	16	18	19	70	1.2	NC
7. I am more willing to express my emotions.	15	14	44	20	44	1.57	CSD
8. I established a new path for my life.	0	29	29	26	53	1.30	NC
9. I know better that I can handle difficulties.	0	68	26	9	34	1.99	CSD
10. I am better able to accept the way things work out.	10	23	23	13	68	1.29	NC
11. I can better appreciate each day.	10	81	13	9	24	2.38	CSD
12. I put more effort into my task.	17	65	19	13	23	2.36	CSD
13. I have a stronger religious faith.	28	62	16	7	24	2.54	CMD
14. I discovered that I'm stronger than thought I was.	15	68	25	7	22	2.43	CSD
15. I learned a great deal about how meaningful my job is.	26	58	26	12	15	2.59	CMD
Grand Mean						**1.63**	**CSD**

Legend:			
	1.51 – 0	NC	Did not experience the change
	2.51 – 1.50	CSD	Experienced the change to a small degree
	3.51 – 2.50	CMD	Experienced the change to a moderate degree
	4.51 – 3.50	CGD	Experienced the change to a great degree
	4.00 – 4.50	CVGD	Experienced the change to a very great degree

Table 2 shows the changes of nurse-respondents as a result of the death of the patient. It is reflected that 7 out of 15 statements are rated by the nurse-respondents as "Experienced change in small degree" or they have experienced the change to a small degree as a result of death. 8 out 15 statements were rated as " No change of experience" But what's significant is the statements "I have a stronger religious faith" and "I learned a great deal about how meaningful my job is" which was rated as "Experienced the change to a moderate degree" this means the moderate change and affect the experience of the nurses. In general the nurse respondents grand mean is equal to 1.63 which means that the nurse-respondents experienced the change to a small degree as a result of the death.

This denotes that there are changes in small degree experienced by the nurse-respondents as a result of patient's death. According to Kubler-Ross(1973) there are changes occurs to a person who is experiencing grief and it involves the five stages of grieving namely; denial, anger, bargaining, depression and acceptance. As shown above that nurses experience mostly positive and showing acceptance and realization to true meaning of their job and their life, it has been captured that most nurse-respondents change experience is the statement "I learned a great deal about how meaningful my job is" which indicates acceptance to the loss and looking forward for positive thought about grief. Thus nurse's grief on patient's death has slight impact on nurse-respondents working life.

Table 3. Contribution of nurse – respondents to the ability to return to functioning following the death of the patient

Statement	4	3	2	1	0	Ave	Interpretation
1. I have found sharing my feelings about the death of my patient.	10	16	43	28	40	1.47	NC
2. I have found that having a support network among my family members and friends.	12	17	47	23	38	1.60	CSD
3. I have found that having supportive people at work, church or other organizations.	12	52	19	8	46	1.83	CSD
4. I have use humour to deal with death.	13	46	46	12	20	2.16	CSD
5. Being able to express my various feelings about death of my patient.	12	14	56	23	32	1.67	CSD
6. Thinking that there are other patients who needs.	25	44	23	23	22	2.22	CSD
7. I understand that its part of my field.	61	23	12	10	31	2.57	CMD
8. Being able to acknowledge the inevitability of death for everyone.	42	42	15	18	20	2.54	CMD
9. Being able to accept the reality of the death of my patients.	20	54	17	14	32	2.17	CSD
10. Being able to feel that I have helped someone else before he/she died.	54	27	26	13	17	2.70	CMD
Grand mean						2.09	CSD

Legend:			
	1.51 – 0	NC	Has not contributed
	2.51 – 1.50	CSD	Contributed the change to a small degree
	3.51 – 2.50	CMD	Contributed the change to a moderate degree
	4.51 – 3.50	CGD	Contributed the change to a great degree
	4.00 – 4.50	CVGD	Contributed the change to a very great degree

Table 3 shows the Contribution of nurse – respondents to the ability to return to functioning following the death of the patient. It is reflected in the result that 6 out of 10 statements were rated as " Contributed in a small degree, 3 out of 10 statements were rated as "Contributed the change to a moderate degree" and 1 out of 10 statements were rated as "Has not contributed". The highest weighted mean is 2.70 on the statement "Being able to feel that I have helped someone else before he/she died." And the grand mean is equal to 2.09 which is interpreted as "Contributed the change to a small degree" or the nurse have contributed in a small degree to progress in the journey following death.

This denotes that there are factors that contribute a small degree of the nurses to return functioning following patient's death. As shown above, mostly that contributed the nurse's ability to return to functioning is having an emotional support from their friends, family or other people. It supports the study of Lani Leary (2012) saying that no one has to be alone when grieving. A person in grief needs a sense of compassionate presence from another person who can "just be" with in whatever way is helpful throughout the journey. Moreover, the statement "Being able to feel that I have helped someone else before he/she died" captured as the majority contributed to return functioning of nurse respondents. This indicates that Filipino Nurses grief ends with acceptance and feeling positive about the things that have done with patient who died. Thus Filipino Nurses experiencing grief to their patients are rapidly resolved when they receive an emotional support from close person and achieve acceptance to the loss and being positive in life.

Table 4. Grief Symptoms of nurse – respondents in relation to the patient who died

Statement	5	4	3	2	1	Ave	Interpretation
1. In the past month, how often you felt yourself longing for everyone for the patient lost?	1	9	12	77	38	1.96	ALO
2. In the past month, how often have you had intense feelings of emotional pain, sorrow, or pangs of grief related to the lost patient?	2	20	1	48	66	1.86	ALO
4. In the past month, how often have you feel stunned, shocked, or dazed by your loss?	2	3	16	28	88	1.58	ALO
5. Do you feel confused about your role in your life?	2	10	15	32	78	1.75	ALO
6. Have you had trouble accepting the loss?	2	2	12	33	88	1.55	ALO
7. Do you feel bitter over your loss?	0	0	13	34	90	1.48	NA
8. Do you feel that moving on from your loss?	8	12	14	34	69	1.99	ALO
9. Do you feel emotionally numb since your loss?	0	0	13	21	103	1.4	NA
10. Do you feel that your life is unfulfilling, empty, or meaningless since your loss?	0	0	14	27	96	1.46	NA
Grand Mean						1.67	ALO

Legend:				
	1.00 – 1.50	1	NA	Not at all
	1.51 – 2.50	2	ALO	At least once
	2.51 – 3.50	3	ALOW	At least once a week
	3.51 – 4.50	4	ALOD	At least once a day
	4.51 – 5.00	5	STD	Several times a day

Table 4 shows the grief symptoms of nurse – respondents in relation to the patient who died. It is reflected in the table that 6 out of 9 statements were as "At least once" or the nurses experience symptoms at least once in a while regarding the present grief symptoms in relation to the patient who died. And 3 out of 9 statements were rated as "not at all". The highest weighted mean belonged to the statement "Do you feel that moving on from your loss?" and the grand mean is 1.67 and rated as "At least once".

This denotes that most of the grief symptoms of Filipino nurses in relation to patient's death is moving on from the loss. It is also indicated that most of the Filipino nurses are longing for the patient they loss, they have feel stunned, shocked or dazed by the loss. As cited by brown and wood (2009), nurses tried to find common ground between the need to remain professional and strong. Thus, the Filipino Nurses try to cope with grief they experienced, to remain strong and still able to work well.

Table 4.1. Grief Symptoms of nurse – respondents in relation to the patient who died

Statement	1	2	Mean	Interpretation
3. For question 1 or 2 above, Have you experienced either of these symptoms at least daily and after 6 months have elapsed since the loss?	129	8	1.06	No

Legend:			
	1.00 – 1.50	1	No
	1.51 – 2.00	2	Yes

For questions 1 or 2, table 4.1 shows that the weighted mean of 1.06 which is rated as "NO" is interpreted as Filipino nurses did not experience the symptoms at least daily and after 6 months have elapsed since the loss. This denotes that majority of the nurses did not experienced prolonged symptoms related to the patient's death. Thus, there is a progress in the grief process of the Filipino Nurses and still they are able to manage their emotions.

7Table 5. Relationship between Nurse - respondents demographic profile and the changes and extent to return functioning experienced as a result of patient's death

Variates	rxy	P-value	Evaluation	Decision
1.Age	0.417	0.002	S	Reject Ho
2. Sex	0.062	0.690	NS	Accept Ho
3. Civil status	0.035	0.690	NS	Accept Ho
4. Employment status	0.144	0.690	NS	Accept Ho
5. Religion	0.175	0.690	NS	Accept Ho
6. Number of years in the hospital	0.345	0.002	S	Reject Ho
7.Number of patient's death	0.064	0.463	NS	Accept Ho

Legend: α = 0.05; P-value=>0.05;NS- Not Significant, S- Significant, df= 33 ** 0.05 level(2 tailed analysis)

As presented in the table, the r- value shows perceived grief of the respondents and age has 0.417 with a corresponding p-value of 0.002of 2 tailed analysis. The r value interpreted as moderate correlation. Since p-value is lesser than 0.05 level, which means that there is significant relationships between the perceived grief of the Filipino Nurse - respondents and their age.

Finally, the r- value shows perceived grief of the respondents and Number of years in the hospital has 0.345 with a corresponding p-value of 0.002 of 2 tailed analysis. The r value interpreted as moderate correlation. Since p-value is lesser than 0.05 level, which means that there is significant relationships between the perceived grief of the Filipino Nurse - respondents and number of years in the hospital.

It denotes that majority of the respondents experience changes as a result of patient death including: changed priorities about what is important in life, developed new interests, can handle difficulties , able to accept the way things work out, appreciate each day, more effort into my task, stronger religious faith, discovered strength, and learned a greta deal about how meaningful the job is. As cited by Zisook and Zisook (2005), other changes in thinking and attitude include reviewing and ranking values, becoming wiser, shedding illusions about immortality, viewing the world more realistically, and reevaluating religious or spiritual beliefs. Thus, the Filipino Nurses are still able to handle situations.

Conclusion:

Grief on patient's death in most of the hospitals in Samar, Philippines is dominated by Females Filipino Nurses. Most of the respondents are Roman Catholic and is single and has mostly one year working experience and has able to witnessed more than 6 patient death. Majority of the respondents experienced the change to a small degree as a result of patient's death. Most of the Filipino Nurses contributed in a small degree of the ability to go back to functioning following patient's death, thus there is a possibility that the Filipino nurses has the high chance to return to normal functioning following patient's death. In general, Filipino nurses experience symptoms at least once in relation to patients death. The Filipino Nurses age and years of working experience has a significant relationship on the grieving process. Results shows that as the Filipino nurse ages and increases their number of years of working experience they are able to easily adapt and accept the grieving process. Therefore the younger the Filipino nurse with short working experience or being novice, the longer the grieving process, on the other hand as the nurse ages and increase their working experience and witnessed more patients death, the more they are adaptive and making it more easy for them to accept the process and making it short. Therefore it is recommended that Filipino nurses should have a strong support group to easily adapt the grieving process. It is also recommended to establish the Nurses grief management program for them to increase their knowledge, skills and attitude towards this process. Also to integrate this program into hospital protocols and regular program to support nurses in this process, their participation will increase their interest in the grief management program at their workplaces.

Acknowledgements

This research study would not be possible without the help of people who humbly and kindly contributed and made time in assisting for the preparation of the completion of this study, sincerest thanks and profound gratitude to all those who made this study possible.

Competing Interest

The author has no competing interests which may have influenced in writing this article.

References

Anderson K., Gaugler J. (2006), The grief experience of certified nursing assistants; Personal growth and complicated grief, Omega, pages 301 – 318

Barrere, C.C., Durkin, A. and LaCoursiere, S. (2008). The influence of end-of-life education on attitudes of nursing students. International Journal of Nursing Education, pages 1-18

Beckstrand, R.L., Callister, L.C., and Kirchhoff, K.T. (2006) Providing a "good death" critical care nurses' suggestions for improving end-of-life care. American Journal of Critical Care, pages 38-45

Beckstrand, R.L., and Kirchhoff, K.T. (2005) Providing End-of-life care to patients: Critical care nurses' perceived obstacles and supportive behaviors. American Journal of Critical Care, pages 395-403

Brajtman, S. Fothergill-Bourbonnais, F., Casey, A., Alain D., and Fiset, V. (2007) Providing direction for change: assessing Canadia nursing students' learning needs. International journal of palliative Nursing, pages 213-221.

Cooper, J. and Barnett, M. (2005). Aspects of caring for dying patients which cause anxiety to first year student nurses. International journal of palliative Nursing, pages 423-430.

Dunn, K.S., Otten, C., and Stephens, E. (2005). Nursing experience and the care of dying patients. Oncology Nursing Forum, pages 97-104.

Kozier, B., Erb, G., (2004) Fundamentals of Nursing practice: Concepts, Process and practice, seventh edition. New Jersey: Pearson Education Incorporated

Lani Leary (2012); No one has to be alone; Psychologytoday.com/blog/no-one-has-bealone/201204/no-one-has-grieve-alone

Lisa Boncheck Adams (2012); Some differences in grieving styles between men and women; lisabadams.com/some-differences-in-grieving-styles-between-men-and women/

Meadors P., Lamson A. (2008) Compassion fatigue and secondary traumatization provider self-care on intensive care units for children journal of pediatric healthcare; pages 24-34

Moores (2007) Memorable patient deaths; reactions of hospital doctors and their need for support Medical Education; pages 41 and pages 942-946

Rickson(2005) how well are we caring for caregivers? Prevalence of grief related symptoms and need for bereavement support among long term care staff. Journal of pain and symptom management, page 227-233.

Ryan, L.A. (2005). The journey to integrate Watson's caring theory with clinical practice. International Journal of Human caring, pages 26-30.

Shiela L. Videbeck (2011); Psychiatric-Mental Health Nursing (Fifth Edition); pages 205-223

Lisa Boncheck Adams (2012); Some differences in grieving styles between men and women; lisabadams.com/some-differences-in-grieving-styles-between-men-and women/

Meadors P., Lamson A. (2008) Compassion fatigue and secondary traumatization provider self-care on intensive care units for children journal of pediatric healthcare; pages 24-34

Moores (2007) Memorable patient deaths; reactions of hospital doctors and their need for support Medical Education; pages 41 and pages 942-946

Rickson(2005) how well are we caring for caregivers? Prevalence of grief related symptoms and need for bereavement support among long term care staff. Journal of pain and symptom management, page 227-233.

Ryan, L.A. (2005). The journey to integrate Watson's caring theory with clinical practice. International Journal of Human caring, pages 26-30.

Shiela L. Videbeck (2011); Psychiatric-Mental Health Nursing (Fifth Edition); pages 205-223

Hypoglycemic Effect of Fenugreek in Prediabetes

Jittisa Hlowchitsieng and Werner Kurotschka*

Master of Science Program in Anti-Aging and Regenerative Medicine,
School of Anti-Aging and Regenerative Medicine,
Mae Fah Luang University, Thailand
**Corresponding author. Email:drkuro@aol.com*

ABSTRACT

Background: Recent epidermiology study has estimated that the prevalence of diabetes is increasing at an extremely higher rate. And diabetes greatly increases the risks of macro- and microvascular diseases. Prevention and treatment of diabetes are not only available with conventional medicine. The herb fenugreek (Trigonella foenum-graecum L., Fabaceae family) is used both in cooking and for the treatment of diabetes in many parts of the world but its efficacy for glycemic control remains unclear. This study aimed to determine fenugreek effect in Prediabetes on levels of plasma glucose.
Objectives: To compare effectiveness of 2.1 gm fenugreek extract with placebo for reduce Glucose levels in Prediabetes.
Methods: Thirty-two prediabetic participants were enrolled in prospective randomized double-blind, placebo-control trial. Participants in each group were prescribed with capsules of fenugreek extract or placebo for eight weeks. Researchers follow up blood glucose levels and medication side effects were evaluated.
Results: Plasma glucose level were significantly reduced in fenugreek extract group but non-significantly reduced in placebo group throughout 8 weeks. Between group comparison of plasma glucose level was found that fenugreek extract group was significantly lower compared with the placebo group. Participants were more satisfied with oral fenugreek extract than placebo. And no adverse reaction was found throughout the 8-week study.
Conclusion: 2.1 gm of Fenugreek extract for eight weeks has significantly effectiveness for reduce plasma glucose compared with placebo in Prediabetes. As a result, fenugreek extract might be considered as an alternative treatment in Prediabetes.

Keywords : Prediabtes/ Blood Glucose level/ Fenugreek extract

Introduction

Recent epidermiology study has estimated that the prevalence of diabetes is increasing at an extremely higher rate. In 2000, the global diabetes prevalence was 171 million people (American Diabetes Association, 2003), and it was estimated in 2006 that the number may rise to approximately 366 million people by the year 2030. However, the actual growing number is much increased, 346 million diabetic patient reported in 2011 (World Health Organization 2011). The national statistic for Thai diabetic patients has also shown a similar pattern

Diabetes greatly increases the risks of macro- and microvascular diseases, with similar proportional effects on disease risk observed in Western and Asian populations. As such, diabetes is likely to be an important determinant of the vascular disease burden in countries such as Thailand, where coronary heart disease has been the leading cause of death for over a decade.

Diabetes does not only have impact against quality of life and physical wellbeing, it also affects patients and their relatives economically. In 2006, The American Diabetes Association has made estimation for the national costs of diabetes in USA in 2002 to be 132 billion US dollars and it's projected to increase to 192 billion US dollars in year 2020 (The American Diabetes Association,2003)

Prevention and treatment of diabetes are not only available with conventional medicine. The herb fenugreek (Trigonella foenum-graecum L., Fabaceae family) is used both in cooking and for the treatment of diabetes in many parts of the world, especially in China, Egypt, India and Middle Eastern countries. In low-income countries, individuals with diabetes often do not have access to appropriate medications due to a lack of financial resources. Fenugreek is a widely used herbal medicine for diabetes, but its efficacy for glycemic control remains unclear. This study aimed to determine fenugreek effect in Prediabetes on levels of plasma glucose.

Objectives

To compare effectiveness of 2.1gm fenugreek extract with placebo for reduce Glucose levels in Prediabetes.

Materials and Methods

Inclusion criteria were Inclusion Criteria 1) People in Thailand 2) No current active disease of gastrointestinal tract, liver, kidney, allergy and diabetes. 3) Participants' physical activity levels are sedentary or moderately active. 4) Participants were subjected to pass blood test of BUN,Cr,AST,ALT fasting blood glucose and CBC, physical examination and medical record screening. All blood test results, except fasting blood glucose, should display value within the normal reference range. The fasting blood glucose were between 100 and 125 mg/dL. 5) Subject voluntarily participated in the study and signed their names in consent forms. Exclusion Criteria 1) History of being allergic or intolerant to Fenugreek products. 2) History of diseases of gastrointestinal tract, liver, kidney or allergy, and other diseases which may influence the bioavailability of fenugreek. 3) History of regular alcohol consumption or drug abuse. 4) History of regular smoking.

Volunteers were screened for non-diabetic condition according to the inclusion and exclusion criteria. Participants provided their personal profile. Participants were explained about the detail and procedures of the study. They willingly signed the informed consent form for clinical trial.

Fasting at least 8 hours, blood samples were collected from all participants for their blood glucose level. Were randomly divide volunteers into 2 groups. Fenugreek or Placebo group.

In Fenugreek group, received 2.1 gm Fenugreek per day.

In control group, received placebo.

After experiment (8 weeks), fasting at least 8 hours, collected blood sample from all participants for measurement of their blood glucose level. All participants kept diary of severity of abdominal and other symptoms rated on a linear scale (0=none through 4=severe) to monitor the adverse effect.

Statistical analysis

Compare Blood Glucose and level before and after experiment in treatment group and placebo group.If information are normal distribution use pair-t –test for statistic analysis. If information are not normal distribution use Wilcoxon signed-ranks test for statistic analysis.

Compare Blood Glucose level between treatment group and placebo group.If information are normal distribution use independent t –test for statistic analysis.If information are not normal distribution use Mann-Whitney U-Test for statistic analysis.

Result

Table 1: Baseline characteristics

	Fenugreek	Placebo	p-value
Age (yr)			
30-39	3(18.8%)	4(25.0%)	
40-49	3(18.8%)	5(31.3%)	
50-59	10(62.5%)	7(43.8%)	
Mean±SD	50.44±7.60	46.19±9.25	0.167
Sex			0.476
Male	6(37.5%)	8(50.0%)	
Female	10(62.5%)	8(50.0%)	

Compare mean age and sex between 2 group by student t-test and chi-square test, respectively

Table 2: Comparison of fasting blood sugar between Fenugreek VS Placebo group and within group compare before VS after treatment

	Before	After	Change
Fenugreek	109.81±8.31	99.38±7.22	10.44±5.72
Placebo	108.31±7.83	107.81±6.75	0.50±5.54
Compare between 2 group			
t,df	0.53,30	-3.41,30	4.99,30
P-value	P=0.603	P=0.002*	P<0.001*

p-value between group from student t-test , p-value within group (before VS after) from paired t-test, *=significant at p<0.05 Change= (before-after), %Change =((before-after)/before)x100,

Figure 1: Comparison of fasting blood sugar between Fenugreek VS Placebo group

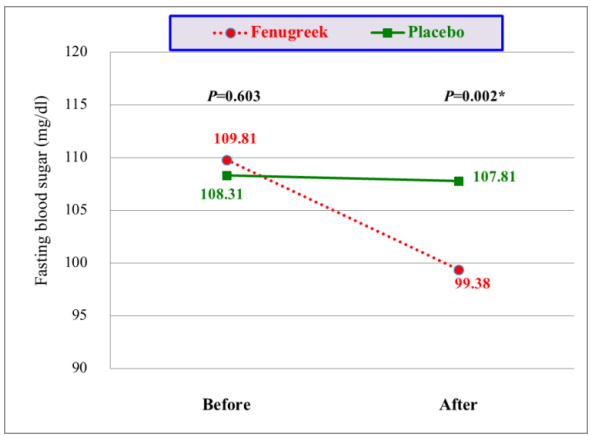


Figure 2: Comparison mean change of fasting blood sugar (before-after) between Fenugreek VS Placebo group

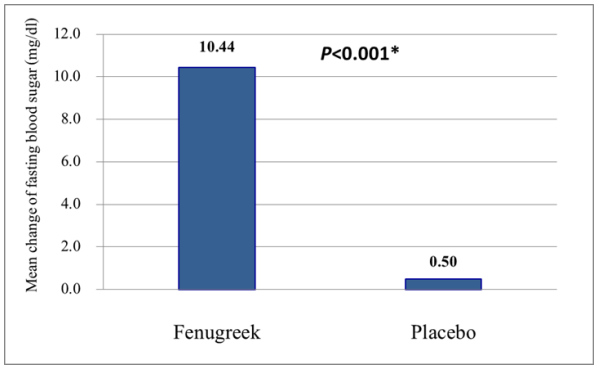


Table 3: Comparison of satisfaction between Fenugreek and Placebo group

	Fenugreek	Placebo	P-value
Moderate	0(0.0%)	1(6.2%)	
High	4(25.0%)	7(43.8%)	
Hightest	12(75.0%)	8(50.0%)	
Mean±SD	3.75±0.45	3.44±0.63	0.130

p-value between group from Mann Whitney U-test

Discussion

The fraction of fenugreek that contains the testa (i.e., the portion of the fenugreek seed with the peculiar smell and bitter taste) and the endosperm of the defatted seeds (i.e., the "A" subfraction) are thought to be associated with the hypoglycemic effects of fenugreek. Fenugreek seeds contain 50-percent fiber (30-percent soluble fiber and 20-percent insoluble fiber) that can slow the rate of postprandial glucose absorption. This may be a secondary mechanism for its hypoglycemic effect. In humans, fenugreek seeds exert hypoglycemic effects by stimulating glucose-dependent insulin secretion from pancreatic beta cells, as well as by inhibiting the activities of alpha-amylase and sucrose two intestinal enzymes involved in carbohydrate metabolism.

The hypoglycemic activity of fenugreek was evaluated in three clinical trials with healthy or healthy obese volunteers. The first early trial (Abdel-Barry JA,2000) recruited 20 male healthy subjects. The treatment group received 40mg/kg aqueous extract of fenugreek seeds whereas the control group received placebo. Four hours post-ingestion, blood glucose levels were significantly reduced in the treatment group. The second trial (Mathern JR, 2009) was a single blind, randomized, crossover study and conducted in 18 healthy obese subjects. Two treatment groups received 4g or 8g of isolated fenugreek fiber whereas the control group received a placebo. No significant changes were noticed in postprandial blood glucose levels and insulin sensitivity within 3.5 hours post-ingestion between the three groups. The third trial (Chevassus et al., 2010) is a double-blind, randomized, and placebo-controlled study with 38 healthy overweight male volunteers. Treatment group with 18 subjects received 1176mg daily dose of hydroalcohol extract of fenugreek seed for 6 weeks while the control group with 20 subjects received placebo. At the end of the study, no significant differences in FBS and insulin were detected between the control and treatment group.

Our results are correlated with previous study which 2.1 gm fenugreek extract can reduce plasma blood glucose compared with placebo within 8th week of study participation.

Further studies are required to evaluate efficacy of long term treatment with or without treatment effects.

Conclusion

2.1 gm of Fenugreek extract for eight weeks has significantly effectiveness for reduce plasma glucose compared with placebo in Prediabetes. As a result, fenugreek extract might be considered as an alternative treatment in Prediabetes.

Reference

Gupta A1, Gupta R, Lal B.: Effect of Trigonella foenum-graecum (fenugreek) seeds on glycaemic control and insulin resistance in type 2 diabetes mellitus: a double blind placebo controlled study. J Assoc Physicians India. 2001 Nov;49:1057-61.

Xue WL1, Li XS, Zhang J, Liu YH, Wang ZL, Zhang RJ.: Effect of Trigonella foenum-graecum (fenugreek) extract on blood glucose, blood lipid and hemorheological properties in streptozotocin-induced diabetic rats. Asia Pac J Clin Nutr. 2007;16 Suppl 1:422-6.

Neelakantan N1, Narayanan M, de Souza RJ, van Dam RM.: Effect of fenugreek (Trigonella foenum-graecum L.) intake on glycemia: a meta-analysis of clinical trials. Nutr J. 2014 Jan18;13:7. doi: 10.1186/1475-2891-13-7.

Chevassus H, Molinier N, Costa E, Galtier F, Renard E, Petit P.: A fenugreek seed extract selectively reduces spontaneous fat consumption in healthy volunteers. Eur J Clin Pharmacol. 2009 Dec,65 (12): 1175-8. doi:10.1007/s00228-009-0733-5. Epub 2009 Oct 7.

Bordia A, Verma SK, Srivastava KC.: Effect of ginger (Zingiber officinale Rosc.) and fenugreek (Trigonella foenumgraecum L.) on blood lipids, blood sugar and platelet aggregation in patients with coronary artery disease. Prostaglandins Leukot Essent Fatty

Acids. 1997;56:379–384. doi: 10.1016/S0952-3278(97)90587-1.

Lu F, Shen L, Qin Y, Gao L, Li H, Dai Y.: Clinical observation on Trigonella Foenum-graecum L. total Saponins in combination with sulfonylureas in the treatment of type 2 diabetes mellitus. Chin J Integr Med. 2008;14:56–60. doi: 10.1007/s11655-007-9005-3.

Alamdari KA, Choobineh S, Jadidi JP.: Antidiabetic effects of exercise and fenugreek supplementation in males with NIDDM. Medicina Dello Sport. 2009;62:315–324.

Raghuram TC, Sharma RD, Sivakumar B, Sahay BK.: Effect of fenugreek seeds on intravenous glucose disposition in Non-insulin-dependent diabetic-patients. Phytother Res. 1994;8:83–86. doi: 10.1002/ptr.2650080206.

Sharma RD, Raghuram TC, Rao NS.: Effect of fenugreek seeds on blood glucose and serum lipids in type I diabetes. Eur J Clin Nutr. 1990;44:301–306.

Sharma RD, Raghuram TC.: Hypoglycemic effect of fenugreek seeds in Non-insulin-dependent diabetic subjects. Nutr Res. 1990;10:731–739. doi: 10.1016/S0271-5317(05)80822-X.

Suksomboon N, Poolsup N, Boonkaew S, Suthisisang CC.: Meta-analysis of the effect of herbal supplement on glycemic control in type 2 diabetes. J Ethnopharmacol. 2011;137:1328–1333. doi: 10.1016/j.jep.2011.07.059.

Mathern JR, Raatz SK, Thomas W, Slavin JL.: Effect of fenugreek fiber on satiety, blood glucose and insulin response and energy intake in obese subjects. Phytother Res. 2009;23:1543–1548. doi: 10.1002/ptr.2795

Yeh GY, Eisenberg DM, Kaptchuk TJ, Phillips RS. Systematic review of herbs and dietary supplements for glycemic control in diabetes. Diabetes Care. 2003;26:1277–1294. doi: 10.2337/diacare.26.4.1277.

Xie W, Zhao Y, Zhang Y. Traditional chinese medicines in treatment of patients with type 2 diabetes mellitus. Evid Based Complement Alternat Med. 2011;2011:726723

Ulbricht C, Basch E, Burke D, Cheung L, Ernst E, Giese N, Foppa I, Hammerness P, Hashmi S, Kuo G. et al.: Fenugreek (Trigonella foenum-graecum L. Leguminosae): an evidence-based systematic review by the natural standard research collaboration. J Herb Pharmacother. 2007;7:143–177.

Faqih AM, Al-Nawaiseh FY.: The immediate glycemic response to four herbal teas in healthy adults. J Med J. 2006;40:266–275.

Bawadi HA, Maghayadah SN, Tayyem RF, Tayyem RF.: The post prandial hypoglycemic activity of fenugreek seed and seeds' extract in type 2 diabetics: a pilot study. Pharmacogn Mag. 2009;4:134–138.

Van Dijk AE, Olthof MR, Meeuse JC, Seebus E, Heine RJ, van Dam RM.: Acute effects of decaffeinated coffee and the major coffee components chlorogenic acid and trigonelline on glucose tolerance. Diabetes Care. 2009;32:1023–1025. doi: 10.2337/dc09-0207.

Identifying the Relationship between Appealing Factors and Perception of Recreational Cycling by 'Generation Y' Corporate Workers in Bangkok Metropolis

Apitsara Tangsathienpan and Apichart Intravisit

Graduate School of Business, Assumption University of Thailand
Corresponding author. Email: apitsara2532@gmail.com or apichart.intravisit@gmail.com

Abstract

Nowadays, being physically active and living a healthy lifestyle has become extremely important for people around the world. People would like to take part in activities that burn large amounts of energy than when they are not active. Cycling, running, and walking have become fashionable again and bicycling for recreation is becoming a major trend in many cultures around the world. This research did a thorough review of literature related to perceptions towards recreational cycling and identified appealing factors based on information available from previously published research. Data was collected from 200 respondents using both paper-based and online surveys. This study found that all four factors have a relationship with perception of recreational cycling in varying degrees. It was found that social factors had the strongest relationship with perception of recreational cycling while health and physical environmental factors had moderate relationships. The final factor, media, was found to have no relationship with perception of recreational cycling. This study can be an important point of reference to cycling activities organizers when they want to learn what factors they should focus on to increase participation at recreational cycling events and activities. This in turn can lead to a more effective impact of their mission and business plan with greater profit maximization.

Keywords: Recreational Cycling, Generation Y, Corporate Workers, Physical activity, Cyclist‟s perception

Introduction

Background

The first bicycle was invented in the 19th century in Europe. Is has become a means of transportation for many people in regions around the world. Bicycles play and important role in daily lives of people from their childhood to late in the lives of Generation Y men and women in the Bangkok corporate community. The main reason behind the invention of the bicycle was for transportation; however, nowadays they are used for many other purposes such as recreation, exercise, sport and competition. Organizations can arrange trips for cyclists or those who are interested in participating in cycling trips to build connections with members and to know and learn about each other to build a new community. Next, cycling can help people live longer and better as people who bike have better insulin levels, weight and blood pressure. Furthermore, cycling can help improve muscles with little strain and strengthen muscles of legs and hips. More than that, cycling has been a competitive sport in the Asian Games since 1951 in New Delhi and it is also included in Olympic Games, the most important sporting event in the world. It means that cycling is an interesting activity.

There are many recreational cycling activities organized in Bangkok Metropolis that invite cyclists from all walks to participate. Some of the really well-known activities are "Bike for Dad" and "Bike for Mom". These events are organized to express gratitude and to show the unity of the people towards our beloved monarch, His Majesty the King Bhumibol Adulyadej, and Her Majesty Queen Sirikit. Hundreds of thousands of Thai and non-Thai cyclists participate in each of these events that are both led by His Royal Highness Crown Prince Maha Vajiralongkorn. More than 500,000 people have participated in both these events in the past years, providing evidence that recreational cycling is a really popular and interesting activity. Based on the reasons above, we can assess that cycling is a popularecreational activity nowadays and the price of purchasing a bicycle can start from a hundred thousand baht up to a million baht. It also means that people can have a bicycle based on their budget for this activity. Generation Y is the group of people who were born between 1981 and 2001 and have a different lifestyle when compared with other generations. They have specific characteristics, for example; they have flexible working time and are able to manage time for other activities in their lives. They are energetic and like to participate in interesting activities and have the purchasing power to purchase things they need. Furthermore, they like to do activities that are in trend in a given period and they also would like to be accepted in their own social groups. The relationship between bicycles and the lifestyle of Generation Y have linkages between each other because cycling is an in trend activity at the moment and Generation Y people have the purchasing power to purchase bicycles and accessories to participate in this recreational and interesting activity. Recently, bicycles have achieved a popular status. They no longer serve as mere economical vehicles, but they also represent health, social status, and a fashionable activity that even represents personal identity.

Statement of Problem

Regarding the topic of identifying the relationship between appealing factors of recreational cycling and the perception of recreational cycling by Generation Y corporate workers in Bangkok Metropolis, this research studies the relationship between appealing factors of cycling and the perception of recreational cycling by Generation Y corporate workers in Bangkok Metropolis by following a framework that incorporates health, media, physical environmental factors and social factors. There are a limited number of studies that have been done pertaining to this area, and it is a very interesting topic to better understand appealing factors that may have a relationship with the perception of recreational cycling. Furthermore Generation Y is becoming a stronger player in the sports products market as this generation has strong purchasing power and social connections, which are interesting characteristics. This research seeks to the answer the following research questions.

Research Questions

1. What constitute as appealing factors of recreational cycling by Generation Y corporate workers in Bangkok Metropolis?
2. To what extent are the appealing factors of recreational cycling related to the Generation Y corporate workers in Bangkok Metropolis‟s perception of recreational cycling?

Research Objectives

1. To describe the appealing factors of recreational cycling for Generation Y corporate workers in Bangkok Metropolis.
2. To identify the extent to which the appealing factors of recreational cycling are related to the Generation Y corporate workers in

Bangkok Metropolis‟s perception towards recreational cycling.

Literature Review

Health

The World Health Organization (WHO) defines health as, a definition which was drawn up in 1948, "state of complete mental, physical and social well-being and not only the absence of infirmity or disease" (WHO, 1986). Nowadays, people are more concerned about their health because good health is very important to live a modern life without disease and to have a better life. Therefore people are more interested

in being healthy and are health conscious.

Media

Media is defined as channels of communication which people apply as a medium to send and receive information such as advertising media, news from television, radio and social media. Media is one of the most influence channels of communication with the mass of people around the world. It can significantly influence people‟s behavior and perception.

Physical Environmental Factors

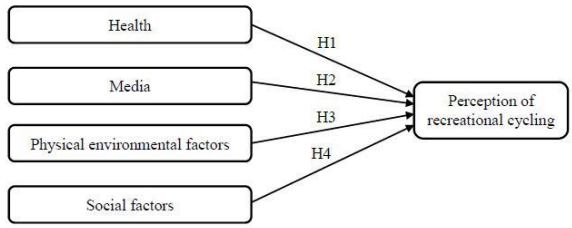
Physical environment is the tangible and material world in which we live. There are four factors which make up physical environment: Function (permeability, streets, traffic and walking surface), Safety (traffic and personal), Aesthetics (views and streetscape) and the last one is Destination, which is facilities. Nowadays, people don‟t consider only the category of activity but they also take into consideration the physical environmental factors before they decide to participate in the activity. **Social Factors**

It is the system of relationship that exists among the individuals of a group (Umar Farooq, 2011). For example; membership club, family. Therefore social factors are the factors which affect perception and influence modern lives of people.

Research Frameworks

Conceptual Framework

Overview of the framework used in this study to explain the relationship between appealing factors which are health, media, physical environmental factors and social factors with perception towards recreational cycling.



Research Hypotheses

There are four proposed hypotheses in this study research as the followings;

$H1_0$: There is no a relationship between health and perception of recreational cycling.

$H1_a$: There is relationship between health and perception of recreational cycling.

$H2_0$: There is no relationship between media and perception of recreational cycling.

$H2_a$: There is a relationship between media and perception of recreational cycling.

$H3_0$: There is no relationship between physical environmental factors and perception of recreational cycling.

$H3_a$: There is a relationship between physical environmental factors and perception of recreational cycling.

$H4_0$: There is no relationship between social factors and perception of recreational cycling.

$H4_a$: There is a relationship between social factors and perception of recreational cycling.

Research Methodology

Methods of Research Used

This research is uses descriptive research method based on type of survey method research which was conducted using questionnaires administered to respondents. After receiving the completed questionnaires and demographic profiles of the sample, this paper will attempt to answer who, what, where, when, which and how questions (Zikmund, 2003). Then, the scaling factor and perception of recreational cycling by Generation Y corporate workers in Bangkok Metropolis are explained. Descriptive research method has been used to study the relationship between appealing factors and perception of recreational cycling by Generation Y Corporate workers in Bangkok Metropolis. The research method used to collect primary data and information from respondents was through the distribution of questionnaires. This method provided a multifaceted approach for data collection and also proved to be accurate, inexpensive, efficient and quick (Zikmund, 2004). The researcher did the sample survey by distributing the questionnaire to respondents who participate in recreational cycling and are among Generation Y corporate workers in Bangkok Metropolis. This study aims to identify relationships amongst health, media, Physical environmental factors and social factors towards perception of recreational cycling. In addition, the Statistical Package for Social Science (SPSS) program was used to analyze data collected from the questionnaires for hypothesis testing.

Respondents and Sampling Procedures

Target populations are cyclists who are in Generation Y Corporate Workers in Bangkok Metropolis. The researcher distributed 200 questionnaires through two distribution channels, paper-based and online survey, and only focused on respondents who are able to ride bicycles and are among Generation Y corporate workers in Bangkok

Metropolis. The researcher used both of purposive sampling and convenience sampling.

Research Instruments/ Questionnaire

The researcher creates questionnaires in order to identify the Relationship between Appealing Factors and Perception of Recreational Cycling by

„Generation Y" Corporate Workers in Bangkok

Metropolis. The questionnaire was composed of three parts including screening questions, variables measurement and personal information.

Conclusions and Recommendations

Summary of finding descriptive analysis

According to analysis of Personal data, most of respondents were male 58% and age is between 26-31 years old 58%. The marital status is single 73% and education level is below bachelor degree 64.5% and their income is between 20,001-40,000 baht 44.5%. The most effective communication channel is Facebook 86% and they had ever attended recreational cycling activity 52.5%. The favorite place to ride recreational cycling is Sky lane 37% and the most favorite bicycle type is Road bike 34.5%, for budget spending for bicycle is 10,001-30,000 baht 53%. Frequency of time spend is less than 5 times per month 60% and the average time spending is 1-3 hours 65% and average distance is less than 10 kilometers per time 95% and they always cycle alone 40.5%.

Table1: Summary of demographic information

Variable	Majority	Total respondents %
Gender	Male	58%
Age	26-31 years old	58%
Marital status	Single	73%
Education level	Below Bachelor degree	64.5%
Income	20,001-40,000 baht	44.5%
Communication channel	Facebook	86%
Recreational cycling participation	Participation	52.50%
Favorite place	Sky lane	37%
Bicycle type	Road bike	34.5%
Budget of spending bicycle	10,001-30,000 baht	53%
Frequency of time spend	Less than 5 times per month	60%
Average time	1-3 hours	65%
Average distance	Less than 10 kilometers per time	95%
Buddy	Alone	40.5%

Summary of results finding Hypothesis testing

The findings on cyclists" perception are based on the hypotheses testing results of the correlation value which shows that some independent variables are significant at 0.000 (sig. 2-tails) at 0.01 level and one variable is not significant. The summary on findings are shown in Table 2.

Table 2: Summary of hypothesis testing

Hypothesis	Statement	Pearson Correlation	Sig. level	Testing Result
H1	There is relationship between Health and perception of recreational cycling	.505	.000	Moderate relationship
H2	There is relationship between Media and perception of recreational cycling	.155	.029	No relationship
H3	There is relationship between Physical environmental factors and perception of recreational cycling	.455	.000	Moderate relationship
H4	There is relationship between Social factors and perception of recreational cycling	.644	.000	Strong Relationship

Conclusion

This research studied the relationship between health, media, physical environmental factors and social factors towards perception of recreational cycling. The researcher used previous studies as a foundation to create a new conceptual framework to examine the relationship between variables. The researcher used a questionnaire as a tool to collect the data and information as primary data to be analyzed. The researcher distributed 200 questionnaires using both paper-based and online method. The researcher collected information from the respondents, who are able to ride bicycle and are Generation Y corporate workers living in Bangkok Metropolis during 10 February to 10 March 2016, as the sample units. The major objective of this research was to study the relationship of health, media, physical environmental factors and social factors towards perception of recreational cycling. To achieve the objectives, hypotheses have been set and tested using the Pearson Correlation Coefficient. Based on this research, this study found the majority percentage of each variable. The demographic variables included gender, age, marital status, education level, income level, the favorite communication channel, the favorite place of recreational cycling, and the type of bicycle, budgeting of bicycle, frequency of riding, average riding time, average distance and buddy who the respondents go with. The researcher found that the majority of respondents were male and aged between 26-31 years. Most of the respondents were single and had an education level below bachelor"s degree. Most of them earned 20,001-40,000 baht per month. From the average mean of standard deviation, this research found that the health is the highest mean followed by social factors, then media and finally physical environmental factors. From the results, it was indicated that four null

hypotheses (H10, H20, H3a and H40) had been rejected by the hypothesis testing
procedure. From the first hypothesis test (H1), the results indicated that the significance (2-tailed test) is equal to .000 which is lower than .01 (0.000<0.01). It means that the null hypothesis was rejected at the .01 significance level. At .505, it means that there is a moderate relationship between health and the perception of recreational cycling. It means these two variables move in the same direction. From the second hypothesis test (H2), the results indicated that the significance (2-tailed test) is equal to .029 which is more than .01 (0.029>0.01). It means that the null hypothesis was accepted at the .01 significant level. At .155, it means that there is no relationship between media and the perception of recreational cycling. It means these two variables move in the same direction. From the third hypothesis test (H3), the result from this hypothesis indicated that the significance (2-tailed test) is equal to .000 which is lower than .01 (0.000<0.01). It means that null hypothesis was rejected at the .01 significance level. At .455, it means that there is a moderate relationship between physical environmental factors and the Perception of recreational cycling. It means these two variables move in the same direction. From the fourth hypothesis test (H4), the results indicated that the significance (2-tailed test) is equal to .000 which is lower than .01 (0.000<0.01). It means that the null hypothesis was rejected at the .01 significance level. At .644, it means that there is a strong relationship between social factors and the perception of recreational cycling. It means these two variables move in the same direction.

Recommendations

From the findings of this research, the analysis of the Correlation Coefficient showed the relationship between independent and dependent variables. Furthermore, the results from the hypothesis testing showed that some hypotheses have moderate or strong or no relationships. For health, there is a significant relationship between health and the perception of recreational cycling. The appealing factors of health can affect the perception of recreational cycling of cyclists to participate in recreational cycling activity. The researcher believes that if organizers who conduct recreational cycling activities follow the recommendations, they can increase positive perception to participate in recreational cycling activity. Organizers can promote activity to attract cyclists to attend by talking about health benefits of recreational cycling, and about how recreational cycling can help to improve people‟s health. For media, there is no relationship between media and the perception of recreational cycling. It is the weakest relationship and organizers should focus on the appealing factors however there is no need to spend large investments to promote recreational cycling using this method. For physical environmental factors, there is a significant relationship between physical environmental factors and the perception of recreational cycling. Physical environmental factors are the one of important appealing factors which can influence perception of recreational cycling for increased participation in the activity. Cyclists consider function, safety, aesthetics and destination, therefore organizer should consider these factors as important criteria when selecting places to conduct cycling activities. For example: they should check if the neighborhood around where the activity is to take place has traffic jams or not, they should ensure there is adequate lighting, whether it is clean or not, and whether or not there are available parking spaces. Organizer should consider the physical environmental factor before selecting places to conduct activities because cyclists consider these factors as factors that can affect their perception of recreational cycling. For social factors, there is a significant relationship between social factors and the perception of recreational cycling. It is the strongest relationship and we should pay attention to this variable. The appealing factors of social factors can affect the perception of recreational cycling of cyclists to participate in recreational cycling activities. The positive relationship between social factors towards the perception of recreational cycling tends to increase the participation rate in recreational cycling activities. Therefore when organizers organize such activities, they should more focus on promoting their activities emphasizing on the social benefits, for example; promoting bicycle club memberships or using the power of word-of-mouth marketing to increase participation rates. They can use marketing strategy by launching campaign to have people participate with family or club members, and cyclists can get more benefits from the activity. Now for the results of perception of respondents who had a positive or a negative perception of this activity. Most of them had a positive perception of recreational cycling, they believed that this activity can improve their health, and they believed that this activity can help them to build social relationships with others and physical environmental factors affected their perception of recreational cycling. However media factors had no relationship with perception of recreational cycling. However, organizers can use other method with which to affect the perception of cyclists. For example: using membership clubs to affect the perception of the cyclists by contacting each bicycle club to promote the activity or building relationships or partnerships with the clubs.

References

Ahmed El-Geneidy, D. P. (2015). Cycling Under Influence: Summarizing the influence of perceptions, attitudes, habits and social environments on cycling for transportation. *International Journal of Sustainable Transportation*, *9*(8), 565-579.

D A Lawlor, A. R. (2003). The challenges of valuating environmental interventions to increase population levels of physical activity: the case of the UK National Cycle Network. *Physical*

activity and population health, 96-101.

Danah m. boyd, N. B. (2008). Social Network Sites: Definition, History, and Scholarship. *Journal of Computer-Mediated Communication*, 210-230.

Delfien Van Dyck, M. T. (2015). Relationship of the Perceived Social and Physical Environment with Mental Health- Related Quality of Life in Middle-Aged and Older Adults: Mediating Effects of Physical Activity. *Social and Physical Environmental Factors and Mental Health*, 1-16.

E. Ellis, M. G. (2007). Physical activity and health: evidence from a study of deprived communities in England. *Journal of Public Health, 29*(1), 27-34.

Emma J Adams, A. G. (2013). Correlates of walking and cycling for transport and recreation: factor structure, reliability and behavioural associations of the perceptions of the environment in the neighbourhood scale (PENS). *International Journal of Behavioral Nutrition and Physical Activity*, 1-15.

Greig, R. (2003). Focus group research on cycling. *Health Promotion Journal of Australia, 14*(2), 144-145.

Jenna R Panter, A. P. (2008). Environmental determinants of active travel in youth: A review and framework for future research. *International Journal of Behavioral Nutrition and Physical Activity*, 1-14.

Jeroen Johan de Hartog, H. B. (2010). Do the Health Benefits of Cycling Outweigh the Risks? *Environmental Health Perspectives, 118*(8), 1109–1116.

John Pucher, R. B. (2010). Walking and Cycling to Health: A Comparative Analysis of City, State, and International Data. *American Journal of Public Health, 100*(10), 1986-1992.

John Pucher, R. B. (2011). Bicycling renaissance in North America? An update and re-appraisal of cycling trends and policies. *Transportation Research Part A*, 451-475.

Lin Yang, A. M. (2010). Interventions to promote cycling: systematic review. *Public Health*, 1-10.

Maibach, E. (2007). The Influence of the Media Environment on Physical Activity: Looking for the Big Picture. *The science of health promotion, 21*(4), 353-362.

Philip Blacka, E. S. (2014). The power of perceptions: Exploring the role of urban design in cycling behaviours and healthy ageing. *Transportation Research Procedia 4*, 68-79.

Postma, E. (2014). A relationship between attractiveness and performance in professional cyclists. *Evolutionary biology*, 1-4.

Simon D.S. Fraser, K. L. (2010). Cycling for transport and public health: a systematic review of the effect of the environment on cycling. *European Journal of Public Health, 21*(6), 738-743.

Terri Pikoraa, B. G.-C. (2003). Developing a framework for assessment of the environmental determinantsof walking and cycling. *Social Science & Medicine*, 1693–1703.

Thomas Götschi, J. G.-C. (2015). Cycling as a Part of Daily Life: A Review of Health Perspectives. *Transport Reviews*, 45-71.

Yan Xing, S. L. (2010). Factors associated with proportions and miles of bicycling for transportation and recreation in six small US cities. *Transportation Research Part D*, 73-81.

In vitro Prebiotic Activity of *Artocarpusaltilis* (Breadfruit) Crude Extract for Selected Species of Lactic Acid Producing Bacteria

GlorianiLaarni E. RMT, MSc.

Emilio Aguinaldo College - Cavite, Congressional East Avenue, Burol Main, City of Dasmarinas, Cavite, Philippines
legloriani@yahoo.com[1]

Abstract

This study is designed to assess breadfruit for its prebiotics potential and in resolving the capacity of pre-selected probiotics dairy lactobacilli to utilize oligosaccharide extracted from breadfruit. Bacteria were grown in MRS broth at 37°C in duplicate. Exponentially growing cells were harvested and inoculated in tubes containing 0.5%, 1% and 2% inulin extract and incubated for 24 hours Samples were taken and analyzed for enumeration of the organisms using total plate count. All the observations were studied in triplicates and data were summarized as mean values and Two-way ANOVA, critical differences, confidence interval, % coefficient of variation (CV), and multiple comparisons were applied to the data using SPSS. Results showed that there is a significant difference (p=.000) among the growth of *L. casei ATCC, L. bulgaricus* and *L. casei* Shirota strain inoculated in MRS with inulin, a promising growth was observed on different lactic acid bacterial colony. *L. case*i ATCC and *L. casei* Shirota exhibited a markably increase in colony count as the concentration of inulin increases. This presumably caused by symbiotic effect of pro and prebiotics on growth in number of colony, which also affirmed the strengthening function of prebiotic inulin.

Keywords: prebiotics; lactobacilli; oligosaccharide; *Artocarpus altilis*

Introduction

The growing awareness on prebiotics and probiotics increase the demand of consumers for food products that support health importance of microorganism in human life and in providing beyond basic nutrition. Prebiotics are food components that selectively stimulate the growth and activity of probiotics (Gibson 2004, Panitantum 2004). These prebiotics are polysaccharides of short chain that can withstand acidic and enzymatic digestion in the small intestine (Gibson 2004, Panitantum 2004, and Thammarutwasik et al 2009). Probiotics are viable living microorganisms caused physical changes in microflora in the intestine that contribute to beneficial effects on human health upon ingested in adequate doses (FAO/WHO 2002, Ogeke et al 2010, Widodo et al 2014). The combination of prebiotics and probiotics in a form of synergism is termed as synbiotics. Synbiont effects of prebiotics and probiotics may ensure maintenance of proper equilibrium of microflora in the gastrointestinal system.

Fruits and vegetables are naturally enriched in polysaccharides such as oligosaccharides and fructooligosaccharide which provide an assortment of protective micronutrients and fibers. Breadfruit, *Artocarpusaltilis*, a typically grown as a backyard tree in the Philippines and often been considered to be a form of seedless breadfruit (Barlow 2000). It is a starchy staple crop commonly eaten in the tropics including Philippines and resembles chestnuts in flavor and texture and good source of protein and minerals (Barlow 2000, Appiah et al 2011). Consumption of this fruit according to several studies may help protect against infection, it has proven to have immense potential for antibacterial property (Chinmay et al 2013). It can reduce low density lipoprotein (LDL) cholesterol (Panitantum 2004), it has antihypertensive (Nwokocha et al 2012), antitubercular, antimalarial and anticancer (Boonphong 2007).

Various strains of lactic acid bacteria are used as probiotics that enhance bio-availability of minerals and vitamins thus increasing the resistance to various infectious diseases, boost immune system, improve absorption and maintain mucosal intergrity (Sanders 2007, Seneretal 2014). Lactic acid bacteria used in food industry include *Lactobacillus acidophilus, L. casei, L. plantarum, L. reuteri, L. rhamnosus, L. delbrueckii, L. salivarus, L. helveticus, L. johnsonii, Lactococcuslactis, Bifidobacteriumlactis, B. longum, B. infantis, B. breve, B. animalis*and *B. bifidum*(Sener 2014) which convert carbohydrates into lactic acid thus prevent the pathogenic bacteria from growing.

This study aimed to assess breadfruit for its prebiotic potential. Specifically to resolve the capacity of pre-selected probiotics dairy lactobacilli to utilize oligosaccharide extracted from breadfruit.

Materials and Methods

The standard *Lactobacilluscasei*was purchased from UP Los Baños. MRS broth (Conda), and culture media were obtained from Local Laboratory Supplier. Composition of MRS medium, without glucose, was (g/L): Peptone (10), Agar (10), Beef extract (8), Sodium acetate 3(H2O) (5), Yeast extract (4), K2HPO4 (2), Tri ammonium citrate (2), MgSO4.7H2O (0.2), MnSO4.4H2O (0.05), Sorbitonmonooleate (1ml), pH - 6.2 ± 0.2. *Lactobacillus casei*Shirota strain was isolated from yakult and *Lactobacillus bulgaricus* was isolated from commercially prepared yogurt.

pH and bile salt tolerance and resistance to digestive enzymes

Bacteria were grown in MRS broth (Conda) at 37ºC in duplicate. Exponentially growing cells were harvested by centrifugation at 12000 x g for 10 min, washed twice with sterile saline (0.85% NaCl, W/V pH 7.0) and re-suspended in saline. Sterile saline of pH 2.0 and pH 7.6 were prepared. The entire procedure was based on methods used by Yadavetal (2014) with modification.

Individual pellet of organism was then inoculated in the saline of pH 2.0 and pH 7.6, respectively. Tubes were incubated for 2h. After 2h, pellets were harvested by centrifugation. Samples of 0h and 2h were taken and analyzed for enumeration of the organisms using total viable count.

Collection and processing of materials

Breadfruit indigenous plant materials were chosen from local market Plant pulps were dried in oven and stored as fine powder. Moisture content of all materials was determined simultaneously till constant weight to ensure complete drying.

Extraction of inulin

Inulin from breadfruit was extracted using water (1:1) in blender and then the mixture was vaporized on water bath until concentrated extract to be achieved. It was filtered by using clothes and then the filtratewas diluted in 40% of alcohol 30%v/v from the total of filtrate volume. The diluted filtrate had been stored in freezer (± -100C) for 18 hours and then removed from freezer and kept in the room temperature (± 2 hours). After 2 hours, the solution was centrifuged (5000 rpm, 15 minutes) and then the precipitate was filtered as a crude inulin. The crude inulin was dissolved exactly by using water and then added with 2% w/v of active charcoal solution. The solution was filtered and then diluted by using 40% of alcohol 30% from a total filtrate volume The cooling and centrifuging of the solution was repeated such as the procedure above until the white precipitate was obtained as a pure inulin. The precipitate was dried at 50ºC for 7 hours.

Determination of inulin content

For inulin content determination in extracts a simple and rapid method was used, based on the fact that vanillin, in presence of concentrated sulfuric acid, forms with inulin a deep red color complex that yields a characteristic adsorption spectrum with a peek at 520 nm. The color complex follows Lambert-Beer law within the concentration of inulin of 20-200mg/L. Readings are made with a V-1100D spectrophotometer. Inulin extract was measured using this formula: Inulin extraction yield (%) = (inulin content × volume of extraction liquid/mass of breadfruit) × 100.

Assessment of in-vitro prebiotic potential

Prebiotic potential was assessed for each concentration of the sample in undigested dried states. The assay was done as per the protocol (Agte*et al.*, 2010, Yadav 2014). Glucose from MRS medium was replaced by 20 mg of respective residue of sample material as the only source of carbohydrate. Inulin and MRS medium containing respective concentration of samples were individually inoculated with 100 ul of 24h old individual culture of 0.4 optical densities (O.D.) and were incubated under partially anaerobic conditions at 37oC. O.D. at 600 nm was recorded at 0, and 24 hour of inoculation as a measure of growth using spectrophotometer (Spectrophotometer V-1100-D). Growth of individual organisms in MRS containing breadfruit extract alone was taken as positive control and compared with the growth in presence of inulin with different concentration (0.5%, 1% and 2%). Growth response for all the probiotics was measured in triplicate for all concentrations of breadfruit and expressed as % inulin.

Statistical analysis

All the observations were studied in triplicates and data were summarized as mean values and standard deviations. Two-way ANOVA, critical differences, confidence interval, % coefficient of variation (CV), and multiple comparisons were applied to the data using SPSS v22. The p values $p<0.05$ were considered as significant.

Results and Discussion

A storage polysaccharide in the form inulin consists of short chain of fructose molecules can be extracted from fruits and vegetables such as major crops including breadfruit. This polymer of polyfructans is very soluble in water. Extraction of inulin from breadfruit was done using water as solvent. Inulin content was determined using a simple and rapid method based on Dobre 2008. Breadfruit total inulin content yielded about 16.67%.

Prebiotic activity using dietary bacteria

Growth response of three different probiotic strains (*Lactobacilli casei* ATCC, *L. casei*shirota and *L. bulgaricus*) was monitored in the presence of inulin extracted from breadfruit using MRS (de Man, Rogosa and Sharpe) broth

Table 1. Growth of Lactic Acid Bacteria

Multiple Comparisons

Dependent Variable No. of Colonies

LSD

(I) Lactic Acid Bacteria	(J) Lactic Acid Bacteria	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
L. casei ATCC	*L. bulgaricus*	-41.875	6.46078	0.000	-54.7985	-28.9515
	L. casei Shirota	-78.5417	6.46078	0.000	-91.4651	-65.6182
L. bulgaricus	*L. casei* ATCC	41.875	6.46078	0.000	28.9515	54.7985
	L. casei Shirota	-63.6667	6.46078	0.000	-49.5901	-23.7432
L. casei Shirota	*L. casei* ATCC	78.5417	6.46078	0.000	65.6182	91.4651
	L. bulgaricus	36.6667	6.46078	0.000	23.7432	49.5901

Based on observed means

The erro term is Mean Square(Error)=500.900

***The mean difference is significant at the .05 level**

Results as shown on table 1, it can be depicted that there is a significant difference (p=.000) among the growth of *L. casei ATCC*, *L. bulgaricus* and *L. casei* Shirota strain. When *Lactobacilli strains* were grown in MRS with inulin extract, a promising growth was observed on different lactic acid bacterial colony.

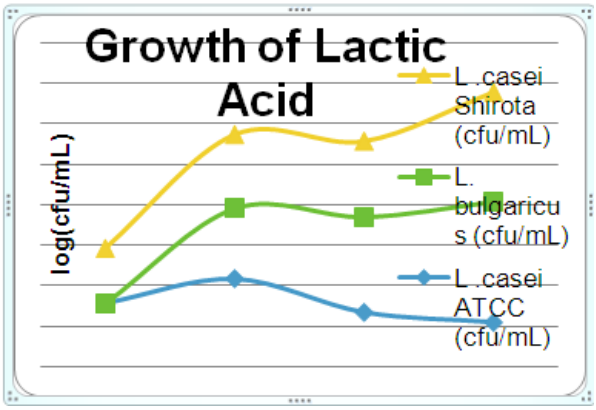


Figure1. Growth of Lactic Acid Bacteria with Inulin Extract

It can be gleaned on figure1 the viability of lactic acid bacteria can be increased by inulin. The growth of *L. casei* Shirota and *L. bulgaricus* was enhanced with 2% inulin while Number of probiotic bacteria in MRS containing inulin after 24 hours incubation. Counts of probiotics bacteria of the sample were significantly different ($p < 0.05$). It can also be seen on table 2, that the viability of lactic acid bacteria can *L. casei* ATCC is enhanced by lower concentration of inulin (0.5%) but the decrease in the concentration from 0.5% to 1% is not significant (p=.140) as shown in table 2. This can be explained that the energy source was depleted after 24 hours of growth, when the cells entered the stationary phase which is comparable to the study conducted by Markas 2005.

Table 2. Inulin Concentration

Multiple Comparisons

Dependent Variable No. of Colonies

LSD

(I) Inulin Concentration	(J) Inulin Concentration	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Control (0%)	*0.5% Inulin*	-188.5556	7.46027	0.000	-203.4783	-173.6328
	1% Inulin	-177.3889	7.46027	0.000	-192.3116	-162.4661
	2% Inulin	-258.2778	7.46027	0.000	-273.2005	-243.355
0.5% Inulin	*0.5% Inulin*	188.5556	7.46027	0.000	173.6328	203.4783
	1% Inulin	11.1667	7.46027	0.140	-3.7561	26.0894
	2% Inulin	-69.7222	7.46027	0.000	-84.645	-54.7995
1% Inulin	*0.5% Inulin*	177.3889	7.46027	0.000	162.4661	192.3116
	1% Inulin	-11.1667	7.46027	0.000	-26.0894	3.7561
	2% Inulin	-80.8889	7.46027	0.000	-95.8116	-65.9661
2% Inulin	*0.5% Inulin*	258.2778	7.46027	0.000	243.355	273.2005
	1% Inulin	**69.7222**	7.46027	0.000	54.7995	84.645
	2% Inulin	80.8889	7.46027	0.000	65.9661	95.8116

Based on observed means

The error term is Mean Square(Error)=500.900.

*The mean difference is significant at the .05 level.

Growth of lactobacilli on prebiotics

Using agar assay, *Lactobacillus* strains grew very well in the presence of oligosaccharides or inulin. Different concentrations of inulin showed significant difference (p=.000) and effect on lactic acid bacteria however, 0.5% concentration when compared with 1%inulin did not show a significant difference (p=.140). Inulin-like fructans are known for their bifidogenic effect or the ability to selectively increase the number of bifidobacteria. In case of *Lactobacillus bulgaricus*which is the largest in size probiotics bacteria, from a physical point of view there cannot be so many cells because of its size.

*L. bulgaricus*is a homofermentative lactic acid and produce D-lactic acid compared to other lactic acid bacteria which produced L-lactic acid. These characteristics ascertain its powerful anti-cancer and anti-oxidant effects. Another important characteristics of *L. bulgaricus*it worked best in association with other probiotics Without Lactobacillus bulgaricus the development of the other probiotic microorganisms is difficult and their positive effects are minimal. It can be stated that the growth of *L. bulgaricus*assayed in this study was stimulated by presence of inulin (Kunova 2011). Since it is a slow growing bacterium and best grow at 72 hours, the increasing concentration of inulin, caused increased in biomass and not in number.

*L. case*i ATCC and *L. casei* Shirota exhibited a markably increase in colony count as the concentration of inulin increases. This presumably caused by symbiotic effect of pro and prebiotics on growth in number of colony. When considering increasing concentration of inulin, the quantity of lactobacilli significantly ($P \leq 0.05$) increased which also affirmed the strengthening function of prebiotic inulin (Kingwatee 2013).

According to DePreter (2011) synbiotic combination (Lactobacillus caseiShirotacells+oligofructose-enriched inulin) indicated a stimulation of saccharolytic fermentation

and, importantly, a reduction of potentially toxic protein fermentation metabolites.

Conclusion

This study showed that lactobacilli are able to utilize the most commonly used prebiotic oligosaccharides such as inulin. In the presence of this prebiotics statistically significant differences in the growth of lactobacilli were observed. It was found that *L. casei*plus inulin had significantly enhanced the growth of lactic acid bacteria

We recommend inulin as a prebiotics additive to dairy products containing lactobacilli. In addition, the isolation and purification of inulin is also suggested prior to probiotics assay and the use of different probiotics from dairy products in evaluation of prebiotics activity is highly recommended.

Acknowwledgement

The proponent would like to thank the following:

1. EAC Cavite Center for Research and Development headed by Dr. Supachai A. Basit for the Financial Grant for this paper.

2. EAC Administration headed by Dr. Georgina B. Palmario, Vice President for Academic Affairs for her unending support.

3. Faculty Members of the School of Medical Technology, Emilio Aguinaldo College - Cavite, Philippines for their support.

References

Agte, V., Khetmalis, N., Nilegaonkar, S., Karkamkar, S., Yadav, S. 2010. Prebiotic potential of juice grape varieties and some hybrids. *J. Sci.Ind. Res.*, 69: 850 854.

Appiah F, Oduro I, Ellis W.O. 2011. AGRICULTURE AND BIOLOGY JOURNAL OF NORTH AMERICA doi:10.5251/abjna.2011.2.5.773.779

AylaSener Mumcu1, Ayhan Temiz2* 2014. EFFECTS of PREBIOTICS on GROWTH and ACIDIFYING ACTIVITY of PROBIOTIC BACTERIA GIDA (2014) 39 (2): 71-77 66375 doi: 10.5505/gida.66375

Barlow, Snow. 2000 Sorting Artocarpus names maintained by Michel H. Porcher/ Multilingual manuscript plant name database/ C 1997-2000. The University of Melbourne.

Breadfruit / Artocarpusaltilis / Diane Ragone Biodiversity InternationalBreadfruit Institute National Tropical Botanical Garden 3530 Papalina Road Kalaheo, Kauai, Hawaii USA 96741 Email: breadfruitinstitute@ntbg.org www.breadfruit.org

C.C. Ogueke, C.I. Owuamanam, N.C. Ihediohanma and J.O. Iwouno. 2010. Probiotics and Prebiotics: Unfolding Prospects for Better Human Health Pakistan Journal of Nutrition 9 (9): 833-843, 2010 ISSN 1680-5194 © Asian Network for Scientific Information, 2010

ChinmayPradhan, MonalisaMohanty, Abhijeeta Rout, AnathBandhy Das, KunjaBihari Satapathy, and Hemanta Kumar Patra. 2013. Phytoconstituent screening and comparative assessment of antimicrobial potentiality of *Artocapusaltilis* fruit extracts. International Journal of Pharmacy and Pharmaceutical Sciences ISSN- 0975-1491 Vol 5, Issue 3, 2013

DePreter, AkliluHabteab Ghebretinsae, Jose Cortinas Abrahantes, Karen Windey, Paul Rutgeerts, Kristin Verbeke. 2011. Impact of the symbiotic combination of Lactobacillus casei shirota and oligofructose-enriched inulin on the fecal volatile metabolite profile in healthy subjects.

Dobre T, Stroescu M, StoicaA ,Draghici E. 2008. Inulin Extraction and Encapsulation.Antohe Chem. Bull. Volume 53(67)1-2

FAO/WHO (2002). Report of a joint FAO/WHO working group on drafting guidelines for the evaluation of probiotics in food. Available from http://ftp.fao.org/es/esn/food/wgreport2.pdf

Gibson, G.R. 2004.Prebiotic. Journal Gastroenterology Supplement. 18(2), 287-298.

Kingwatee, N., 1 Apichartsrangkoon, A., 2*Chaikham, P., 1 Pankasemsuk, T. and 1 Changrue, V. 2013.Survivability and metabolic activity of Lactobacillus casei 01 incorporating lychee juice plus inulin under simulated gastrointestinal environment. International Food Research Journal 21(1): 83-89 (2014) Journal homepage: http://www.ifrj.upm.edu.my

Kunova Gabriela, RadaVojtech, Losova Ivana, RockovaSarka and Vlkova Eva. 2008. In vitro Fermeability of PrebioticOligosaccharidesby Lactobacilli. Vol. 29, 2011, Special Issue: S49–S54

MakrasLefteris, Van Acker Gerald, and De Vuyst Luc. 2005. Lactobacillus paracasei subsp. *paracasei* 8700:2 Degrades Inulin-Type FructansExhibiting Different Degrees of Polymerization. American Society of Microbiology

MaywanHariono1 ,MuhamadFaizin Akbar2 , Indah Sularsih2 ,Lailatun Najihah2 , SarosaPurwadi2 ,ArsaWahyu

Nugrahani. 2009. Extraction, Identification and Acetylation of Inulin From Dahlia Tuber ((Dahlia Pinata Cav.) National Symposium on Ploymeric Materials (NSPM).

Nwokocha CR, Owu DU, McLaren M, Murray J, Delgoda R, Thaxter K, McCalla G, Young L. 2012.Possible mechanisms of action of the aqueous extract of Artocarpusaltilis (breadfruit) leaves in producing hypotension in normotensive Sprague-Dawley rats. / Pharm Biol.2012 Sep;50(9):1096-102. / doi: 10.3109/13880209.2012.658113.

Panitantum, V. 2004. The story of probiotics, prebiotics&synbiotics. A seminar presentation at Kasetsart University, Bangkok, under the auspices of BIOTEC, National Science and Technology Development Agency, Bangkok, Thailand, January 26, 2004, 157-161.

Sanders, M.E., 2007. Probiotics, strain matter Functional foods and neutraceuticals magazine, pp: 36-41.

SPC 2006 Healthy Pacific Lifestyle Section – Secretariat of the Pacific Community (SPC) – BP D5 NoumeaCedex 98848 New Caledonia

Supriya A. Yadav ,Snehal S. Gite, Vikram B. Lanjekar, Smita S. Nilegaonkar* and Vaishali V. Agte . 2014 *In vitro* screening of indigenous plant materials for prebiotic potential. *Int.J.Curr.Microbiol.App.Sci*(2014) 3(11) 137-150 *ISSN: 2319-7706* Volume 3 Number 11 (2014) pp. 137-150

SuratBoonphong, ApiwatBaramee, Prasat Kittakoop , and PakawanPuangsombat. 2007. Antitubercular and AntiplasmodialPrenylated Flavones from the Roots of Artocarpusaltilis /. Chiang Mai Journal of Science, Vol 34, No 3, Sept 2007.

Thammarutwasik, P., Hongpattarakere, T., Chantachum, S., Kitroongrote, K., Itharat, A., Reanmongkol, W., Tewtrakul, S. and Ooraikul, B. 2009.Prebiotics-A review.Songklanakarin J. Sci. Technol. 31 (4), 401-408, Jul. - Aug. 2009

Tuohy KM, Rouzaud GCM, Brück WM, Gibson GR. 2005. Modulation of the human gut microflora towards improved health using prebiotics- Assessment of efficacy.*Curr Pharm Des*, 11: 75-90.

De Preter, AkliluHabteabGhebretinsae, José CortinasAbrahantes, Karen Windey, Paul Rutgeerts, Kristin Verbeke. 2011. Impact of the synbioticcombination of Lactobacillus caseishirota and oligofructose-enriched inulin on the fecal volatile metabolite profile in healthy subjects.MolNutr Food Res 2011 May 31;55(5):714-22. Epub 2011 Jan 31.

Widodo, Anindita N.S., Taufiq T.T., Wahyuningsih T.D (2014). Evaluation of Two *Lactobacillus* Strains as Probiotics with Emphasis in Utilizing Prebiotic Inulin as Energy Source. Int. Res. J. Microbiol. 5(3):33-40

Salting-out Liquid-liquid Extraction (SALLE) Sample Preparation for Quantitative Mass Spectrometry Analysis of Bisphenol A-glucoronide in Human Hrine

Kwanchanok Sareekit[1], Viroj Boonyaratanakornkit[2] and Siriporn Sangsuthum*[2]

[1] Graduate Program in Clinical Biochemistry and Molecular Medicine, Department of Clinical Chemistry, Faculty of Allied Health Sciences, Chulalongkorn University, Bangkok, Thailand.
[2] Department of Clinical Chemistry, Faculty of Allied Health Sciences, Chulalongkorn University, Bangkok, Thailand.
Corresponding author. Email: siriporn.sa@chula.ac.th

Abstract

Bisphenol A is an endocrine disrupting chemicals (EDCs) which acts as xenoestrogen, and may be found in everyday products. The assessment of BPA exposure is the quantitation of major metabolized of BPA (BPA-G) in urine. In this study, we developed a salting-out assisted liquid/liquid extraction (SALLE) method for detecting BPA-G by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The results represented efficiency of SALLE in terms of type and volume adding of reagents in processes to extract BPA-G in urine and successfully in chromatographic condition. The standard curve had a good linear regression with R2 is higher than 0.99. The intraday and interday precisions for the quantitative analysis of BPA-G less than 5%. The LLOD and LLOQ depended on LC-MS/MS was 0.26 ng/mL and 0.87 ng/mL, respectively. This method is useful for BPA-G determination in human urine and the advantages are rapid, short time preparation and not interfere by environmental BPA or other laboratory devices.

***Keywords*:** BPA-G, BPA, SALLE, LC-MS/MS, Bisphenol A- glucuronide

Introduction

Bisphenol A (2,2-Bis(4-hydroxyphenyl) propane, BPA) is a synthetic chemical which worldwide production more than 10^9 kg per year. BPA can be divided in two groups, the first group is used for form polymer, mainly are polycarbonate plastic and epoxy resin. The second group is additives to materials such as thermal paper and polyvinylchloride. For polycarbonate plastic which is synthesized by condensation of BPA and phosgene in order to temperature resistance, transparency and decrease of scratching. Also BPA is precursor of epoxy resins which usually coating inside food packaging materials such as canned food and beverages in order to corrosion resistance of the can from foodstuffs inside, thermal stability, increasing strength [1-3], and prevention of undesirable interaction between the metal from the can and the food. Human expose to BPA from everyday products e.g. food, bank note, thermal paper, etc. Toxicity of BPA correlates with various diseases such as diabetes, cardiovascular disease [4], promotion of proliferation of breast cancer cell line [5] and congenital developmental defects [6].

BPA enter human body through oral, breath, and skin. The major exposure route of BPA is oral administration. The main detoxification process of BPA in human body is glucuronidation process which changes BPA to BPA-glucuronide (BPA-G) and excreted into urine with half-live about 6 h [7, 8]. Thus, the direct assessment of BPA exposure is the determination of BPA-G level in urine. The method for determination of BPA-G can be classified into two methods, enzymatic and non-enzymatic hydrolysis. The enzymatic hydrolysis method is a complicated method from sample preparation steps including enzymatic hydrolysis, extraction, clean up or derivatization step lead to BPA contamination [9]. Total BPA concentration is obtained after the hydrolysis process, the concentration of BPA-G is calculated from the BPA concentration after hydrolysis extracted with the BPA concentration before hydrolysis. Contrast to the non-enzymatic hydrolysis method which directly detect the BPA-G using isotope dilution method base on tandem mass spectrometry (MS/MS), which diminish the hydrolysis process. Resulting to rapid determination of BPA-G with the reliable results.

The standard method for BPA or BPA-G determination is liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) or gas chromatography couple with tandem mass spectrometry (GC-MS/MS). The advantages are high reliable and sensitivity, however sample preparation is a critical step [10]. The extraction process using solvent extraction or solid-phase extraction (SPE) is the most commonly used and most effective methods for the extraction of BPA in food and biological samples. SPE is a high cost

preparative method whereas solvent extraction or liquid-liquid extraction is the high consumable solvent method. The conventional liquid-liquid extraction method base on the different solubility of analyses between immiscible solvents (polar and non-polar). The aim of this study is method development for BPA-G extraction via salting-out liquid-liquid extraction (SALLE). SALLE is the method that an inorganic salt is added into a mixture of water and water-miscible organic solvent leading to a two-phase system separation (aqueous and organic solvent phases). The advantages of SALLE are short sample preparation time, low of reagents using and low cost different from conventional method (e.g. LLE, SPE) [11].

Materials and Methods

Chemicals and reagents

Bisphenol A β-D-glucuronide (BPA-G) and Bisphenol A-$^{13}C_{12}$ β-D-glucuronide ($^{13}C_{12}$BPA-G) were purchased from Toronto Research Chemicals (Toronto, Canada). Water was obtained from a Millipore water purification system (Merck Millipore, Darmstadt, Germany). Acetonitrile (ACN) was HPLC grade from Honeywell Burdick and Jackson (MI, USA). Ammonium acetate was obtained from Emsure (Darmstadt, Germany).

Standard solutions preparation

BPA-G and $^{13}C_{12}$BPA-G were prepared in a pool of urine sample. The concentration of standard BPA-G ranges from 0 ng/mL, 1 ng/mL, 5 ng/mL, 10 ng/mL, 20 ng/mL, 30 ng/mL to 50 ng/mL. Each concentration contained with 20 ng/mL of internal standard (IS), $^{13}C_{12}$BPA-G. All the solutions were stored at -70 °C until used.

Salting-out liquid-liquid extraction (SALLE)

This study, the salting-out liquid-liquid extraction (SALLE) method adapted from Venisse et al. [11] was used to extracted BPA-G from other interference compounds in urine. Each standard urine sample was extracted duplicate by the following method. Acetonitrile 400 µl was added to 200 µl of standard urine sample, mixed for 30 seconds, then added 10 M ammonium acetate 100 µl and mixed 30 seconds. The sample was centrifuged at 15000 rpm for 5 minutes. Three hundred microliters of upper layer was blown to dryness using a nitrogen flow. The Bisphenol A β-D-glucuronide extract was reconstituted with 1 mM ammonium acetate 200 µl and analyzed by LC-MS/MS.

LC-MS/MS analysis

The Agilent 1200 Infinity LC (Agilent Technologies, USA) was directly interfaced to QTRAP 5500 series (SCIEX, USA). The chromatographic separation were performed in a kinetex® C18 column, 50 x 2.1 mm i.d., 2.6 µm particle size (Phenomenex), using ACN/1 mM ammonium as the mobile phase with gradient elution, a flow rate at 200 µL/min, an injection volume of 5 µL, and a temperature at 35 °C. The table of chromatographic condition represented in **Table 1**.

Table 1: The gradient elution program of LC-MS/MS analysis.

Min	1 mM Ammonium Acetate (Mobile Phase A)	% ACN (Mobile Phase B)
0.00	65	35
1.00	65	35
2.00	0	100
9.00	0	100
10.0	65	35
15.0	65	35

The MS/MS parameters were equipped with an electrospray ionization (ESI) in negative ionization multiple reaction monitoring (MRM) mode. For BPA-G, the transition of $[M-H]^-$ m/z was 403 in Q1 to m/z 113 in Q3, all channels were monitored with a 200 ms dwell time. The declustering potential was -57 V; entrance potential was -5 V; collision energy was -21.7 V; the collision cell exit potential was -11 V. The monitor of $^{13}C_{12}$BPA-G, the transition m/z was 415 in Q1 to m/z 239 in Q3. The declustering potential was -105 V; entrance potential was -4.2 V; collision energy was -36 V; the collision cell exit potential was -12 V.

Linearity, LLOD, and LLOQ

The calibration curve was constructed using Analyst® software. (AB SCIEX) and obtained after plotting seven data points of peak area ratio of ions m/z 227 to 239. The concentration of BPA-G were 0 ng/mL, 1 ng/mL, 5 ng/mL, 10 ng/mL, 20 ng/mL, 30 ng/mL and 50 ng/mL. The standard set was analyzed by LC/MS-MS triplicate per standard level and calibration curve was constructed daily. Linearity of calibration curve was determined using least-squares linear regression analysis. Blank tests (30% ACN) were analyzed every extraction batch confirming an absence of BPA contamination between sample run.

The lower limit of detection (LLOD) and lower limit of quantification (LLOQ) depended on LC-MS/MS were calculated with signal-to-noise ratio 3 and 10, respectively.

Accuracy and precision

The accuracy and precision of extracted BPA-G using SALLE method were analyzed triplicate per one extracted sample and calculated % coefficient of variation (%CV) of BPA-G concentrations. The validation of intraday and interday precision, %CV was calculated from the calibration curve at three concentrations, 5, 20, and 50 ng/ml. Interday precision, average %CV was calculated all of two different days.

Results and Discussion

Method development

The standard method for quantitation of BPA-G in human urine is LC-MS/MS. But the critical step is the sample preparation. Solid phase extraction (SPE) is often used for extraction of BPA-G. However, SPE is a high cost preparative method, thus we tried to develop the salting-out liquid-liquid extraction (SALLE) method for extraction BPA-G from urine. The optimized condition was obtained and explained in materials and methods section. After the spiked urines were extracted using SALLE method, the extracted BPA-G was quantified by LC-MS/MS which performed using a C18 column (50 x 2.1 mm, 2.6 µm) and gradient elution. The retention time of both BPA-G and $^{13}C_{12}$BPA-G were around 0.73 minute, under the optimized conditions. The MRM mode in tandem mass spectrometry was used for quantitation of BPA-G. The specific of precursor-product transition for BPA-G (403>113 m/z) and $^{13}C_{12}$BPA-G (415>239 m/z) were set. The specific product ions of BPA-G and $^{13}C_{12}$ BPA-G were selected from the product ion scanning mass spectrum of them (**Figure 1)**.

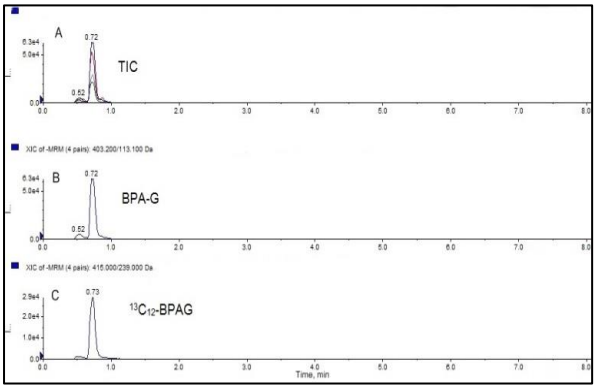


Figure 1: Product ion scanning mass spectrum of BPA-G (403 m/z, $[M-H]^-$) (A) and $^{13}C_{12}$BPA-G (415 m/z, $[M-H]^-$) (B).

The chromatogram of a studied in standard urine sample spiked with $^{13}C_{12}$-BPAG at 20 ng/mL was represented in **Figure 2.** There were no peak of interfering compounds was observed in the chromatogram.

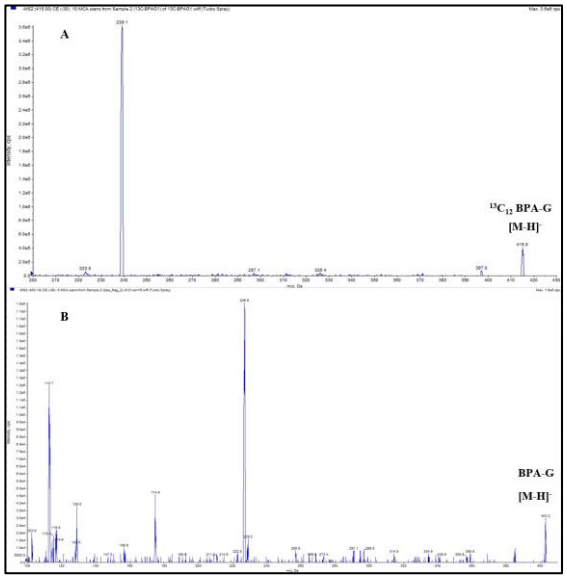


Figure 2: (A) Total ion chromatogram (TIC) for BPA-G and $^{13}C_{12}$BPA-G 20 ng/mL of standard urine sample, (B) Extracted ion chromatogram (XIC) of BPA-G in sample, and (C) Extracted ion chromatogram (XIC) of $^{13}C_{12}$BPA-G 20 ng/mL in sample.

Linearity, LLOD, and LLOQ

The matrix matched calibration curve of BPA-G which was constructed from the area ratio ions m/z 227 to 239 was shown in **Figure 3**. The method showed a good linearity of calibration curve with r^2>0.99. For the quantitation of BPA-G in persons who expose to BPA in further study. The quantitation of BPA-G will be calculated from the calibration curves obtained after addition of known amounts of BPA-G to urine samples from the same subjects collected before BPA exposure [12].

The LLOD depended on LC-MS/MS was 0.26 ng/mL and LLOQ was 0.87 ng/mL. The blank test in this analysis system showed no free BPA contamination in whole experimental processes.

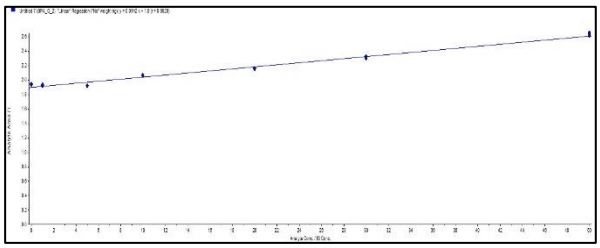


Figure 3: The calibration curve of BPA-G (n=7) constructed from the SALLE preparation and LC-MS/MS.

Accuracy and precision

The accuracy of the method was calculated from the percentage difference between nominal value and average measured value at low (5 ng/ml), medium (20 ng/ml) and high levels (50 ng/ml) of BPA-G. The accuracy ranged from 76.6 to 105.5% (**Table 2**).

The intraday and interday precision for the quantitation analysis of BPA-G were ranged from 0.4 to 1.1, and 4.1 to 4.8, respectively (**Table 2**). The results represented acceptable accuracy and excellent precision method.

Table 2: The intraday and interday accuracy and precision for the quantitation analysis of BPA-G

Intraday (n=2)				
BPA-G	Nominal value (ng/mL)	Average measured value	Accuracy	CV (%)
Low	5	3.9	78.7	1.1
Medium	20	17.2	85.8	1.1
High	50	52.3	104.5	0.4
Interday (n=2)				
BPA-G	Nominal value (ng/mL)	Average measured value	Accuracy	CV (%)
Low	5	3.8	76.6	4.6
Medium	20	16.4	81.7	4.8
High	50	52.8	105.5	4.1

Conclusions

The optimized condition for BPA-G extraction via SALLE was obtained. The method represented short sample preparation time, low of reagents using and low cost different from conventional method (e.g. LLE, SPE). Additional, SALLE method give high selectivity, specificity and efficiency of extraction process. The developed method will may be applied to use for BPA-G determination in human urine in further study.

Acknowledgments

This study was supported by grants from Graduate School Thesis Grant, Chulalongkorn University (GCUGR1225573056M). The authors gratefully knowledge Dr. Piboon Pornmanee, Field Application Specialist from SCIEX for his assistance, and the Halal Science Center, Chulalongkorn University for providing facilities and instruments.

References

[1] Doerge, D.R., Twaddle, N.C., Vanlandingham, M., Brown R.P., and Fisher, J.W. (2011). Distribution of bisphenol A into tissues of adult, neonatal, and fetal Sprague–Dawley rats, Toxicology and Applied Pharmacology, 255(3), 261-70.

[2] Noonan, G.O., Ackerman, L.K., Begley, and T.H. (2011). Concentration of bisphenol A in highly consumed canned foods on the US market, Journal of agricultural and food chemistry, 59(13), 7178-85.

[3] Geens, T., Goeyens, L., and Covaci, A. (2011). Are potential sources for human exposure to bisphenol-A overlooked?, Int J Hyg Environ Health, 214(5), 339-47.

[4] Krishnan, K., Gagne, M., Nong, A., Aylward, L.L., and Hays, S.M. (2010). Biomonitoring Equivalents for bisphenol A (BPA), Regul Toxicol Pharmacol, 58(1), 18-24.

[5] Song, H., Zhang, T., Yang, P., Li, M., Yang, Y., and Wang, Y. (2015). Low doses of bisphenol A stimulate the proliferation of breast cancer cells via ERK1/2/ERRγ signals, Toxicology in Vitro, 30(1), 521-8.

[6] G., Cosimato, C., and Sardo, A.D.S. (2015). Bisphenol A and congenital developmental defects in humans, Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 774 33-9.

[7] Geens, T., Aerts, D., Berthot, C., Bourguignon, J-P., Goeyens, L., and Lecomte P. (2012). A review of dietary and non-dietary exposure to bisphenol-A, Food and Chemical Toxicology, 50(10), 3725-40.

[8] Fisher, J.W., Twaddle, N.C., Vanlandingham, M., and Doerge, D.R. (2011). Pharmacokinetic modeling: prediction and evaluation of route dependent dosimetry of bisphenol A in monkeys with extrapolation to humans, Toxicology and applied pharmacology, 257(1), 122-36.

[9] Harthé, C., Rinaldi, S., Achaintre, D., de Ravel, M.R., Mappus, E., and Pugeat, M. (2012). Bisphenol A-glucuronide measurement in urine samples, Talanta, 100 410-3.

[10] Gallart-Ayala, H., Moyano, E., and Galceran, M.T. (2011). Fast liquid chromatography- tandem mass spectrometry for the analysis of bisphenol A-diglycidyl ether, bisphenol F-diglycidyl ether and their derivatives in canned food and beverages, J Chromatogr A, 25(12), 1603-10.

[11] Venisse, N., Grignon, C., Brunet, B., Thévenot, S., Bacle, A., and Migeot, V. (2014). Reliable quantification of bisphenol A and its chlorinated derivatives in human urine using UPLC–MS/MS method, Talanta, 125 284-92.

[12] Volkel, W., Bittner, N., and Dekant, W. (2005). Quantitation of bisphenol A and bisphenol A glucuronide in biological samples by high performance liquid chromatography- tandem mass spectrometry, Drug Metabolism and Disposition, 33:1748–1757.

The Competitive Efficacy of Biotin and Brewer Yeast vesus Biotin for Glyemic Control in Prediabetes

AlisaDejamornthan[1], Karnt Wongsuphasawat[1]*, Thamthiwat Nararatwanchai[1], Somboon Roongphornchai[1], Rassapoom Sumaetheiwit[2], Atchima Suwanchinda[2],Tanomkit Pawcsuntorn[2] and Phaisal Rummaneetorn[2]

[1]Department of Anti-Aging and Regenerative Medicine, School of Anti-Aging and Regenerative Medicine, Mae Fah Luang University, Bangkok, Thailand
[2]Department of Dermatology, School of Anti-Aging and Regenerative Medicine, Mae Fah Luang University, Bangkok, Thailand
**Corresponding author, Email: karnt.wong@mfu.ac.th*

Abstract

This study aimed to compare the fasting blood glucose-lowering effects of the combination of Biotin and Brewer yeast versus Biotin alone in prediabetes. 30 participants with fasting blood glucose(FBG) 100-125 mg/dL had divided into 2 groups by randomization. The experimental group had been received Biotin 2400 microgram/day along with Brewer Yeast 2000 milligram/day as they split into 2 meals a day for 12 weeks. Controlled trial had been received Biotin 2400 microgram/day split into 2 meals a day for 12 weeks. The comparative Fasting blood glucose-lowering effects of the experimental group (Biotin plus Brewer Yeast) versus the control group (Biotin alone) had tendency of fasting blood glucose level to be decreased in 4 times measuring by p = 0 .81 , 0 .44 , 0 .58 and 0 .6 6 ,respectively. And when considering duration of time found that duration of time taking Biotin and Brewer Yeast had effect on FBG .The experimental group had FBG level decreasing significantly since 4th weeks by p = 0.03. About the control group found that the FBG level decreased only in the 8th weeks by p = 0.02.When considering LDL cholesterol, in the experimental group decrease significantly at p = 0.04 while increase in control group but without statistic significant by p = 0.13. From this study , Both group can reduce FBG level in prediabetes but the experimental group (Biotin plus Brewer Yeast) can reduce FBG faster than the control group (Biotin alone) significantly.

Keywords: *Biotin; Brewer Yeast; Fasting Blood Glucose level; Prediabetes*

Introduction

The prevalence of type 2 diabetes is increasing in THAILAND and worldwide (Mathers and Loncar, 2006). Diabetic patients were predicted to increase to 336 millionin 2030 by The World Health Organization (WHO)(Global status report on non-communicable diseases 2010, 2011). In 2020, the trend of diabetic patients was estimated to increase to a total of 8,200,000 diabetic patients according to the Bureau of Non-Communicable Diseases of Thailand .Insulin resistance, a major causative factor for the early development of type 2 diabetes and cardiovascular disease (CVD), is even more widespread . In addition, there is an increasing prevalence of adult and childhood obesity that markedly contributes to the development of type 2 diabetes (WHO,2014). Although pharmacological options for the management of insulin resistance and type 2 diabetes individuals have been increasing, not all patients have benefited, as the cost and adverse effects of new pharmacologic agents preclude their use in many patients. Though a majority of diabetic patients are being treated, many patients are unable to achieve the currently recommended goal of HbA1c <7%, especially those who are obese (Global status report on non-communicable diseases 2010, 2011). Type 2 diabetic patients are likely to be the most insulin resistant and, therefore, the most difficult to control with currently available standard therapies. Thus, there is a need to identify and evaluate adjunctive therapies that aresafe, efficacious, and cost-effective (Mathers and Loncar, 2006).

Prediabetes is characterized by the presence of blood glucose levels that are higher than normal but not yet high enough to be classed as diabetes. Prediabetes may be referred to as impaired fasting glucose (IFG), or as impaired glucose tolerance (IGT). Prediabetes is often described as the "gray area" between normal blood sugar and diabetic levels and thus have a high risk for developing type 2 diabetes(Global status report on non-

communicable diseases 2014, 2012)Without any medical intervention,Prediabetes patients are more risk to become diabetic patient compared with people who have normal blood glucose level by 3 times within 10 years. However ,the incidence of type 2 diabetes can reduced by control blood glucose level within normal range in prediabetes by adapt their nutrition ,exercise,weight control, promote lifestyle modification (Global status report on non-communicable diseases 2010, 2011) Treatments of type2 diabetes that approved

by FDA are antidiabetic medication in oral form such as Sulfonyluria,Metformin,Glipizide and insulin injection.One adjunctive therapy commonly used by patients to manage their type2 diabetes is natural supplement . Althogh the natural supplements such as

Biotin, Brewers's yeast, Fenugreek are not approved byFDA for diabetic treatments, many previous studies have shown that any supplements such asBiotin,Chromium,Beta-glucan,Fenugreek can decrease fasting plasma glucose in patients with type 2 diabetes(Roglic *et al.*,2005)

The previous study showed Biotin and chromium picolinate which is rich content in Brewers' Yeast significant effect to lowering blood glucose level. The present pilot study was conducted to determine if supplementation with chromium picolinate and biotin can improve glycemic control in patients with type 2 diabetes with suboptimal glycemic control despite use of oral antihyperglycemic agents (WHO,1999). The study of Effects of Chromium and Yeast Supplements on Carbohydrate and Lipid Metabolism in Diabetic reported that type 2 diabetic patients who got Brewers'Yeast as supplement had reduce their FBG level(Roglic *et al.,* 2005).

In 2012 ,The research Combination of Chromium and Biotin Supplementation in Glycemic Control in Prediabetes found Chromium and biotin play essential roles in regulating carbohydrate metabolism and shown that the combination of chromium and biotin significantly decreases FBG in patients with type 2 diabetes compared to the using of chromium alone (Roglic *et al.*,2005). However,the reports of Biotin compare with Brewers' Yeast effects in prediabetes patients is still less study .

Objective

The objective of this study is to compare the fasting blood glucose-lowering effects of the combination of Biotin and Brewer yeast versus Biotin alone in prediabetes.

Materials and Methods

Population

This study included patients who were circumference in Bangkok, male and female and aged between 25-50 years old. Their fasting blood glucose had in range of 100-125mg/dl. All patients came to Prempracha MedicalClinic,Bangkok, during February 2015 to June 2015.

Sample Size Determination

Sample size was defined by the following formula

$$n = \frac{(\quad)^2 \quad ^2 \quad ^2}{(\quad - \quad)^2}$$

$\alpha = 0.05$, $Z_\alpha = 1.645$, d = 15 n = number of samples

= 1.96 (statistics under normal curve; Set $\alpha = 0.05$)

=1.282 (statistics under normal curve; set power analysis =90%and $\beta = 0.1$)

Set confident interval at 95 %

$\alpha = 0.05$

$_1 \ _2$ = the variance and standard deviation of the experiment group and the control group = $_1 \ _2$

$\mu_1\mu_2$ = mean of fasting blood glucose change between the experimental and the control group

Sample size determination was defined from the study of Chromium picolinate and biotin combination improves glucose metabolism in treated, uncontrolled over weight to obese patients with type2 diabetes. (Albarracin, 2007). This study found that $\mu_1 = 9.8 \pm 8.5$, $_2 = 0.7 \pm 5.9$

$$n = \frac{(1.96+1.282)^2 \times (8.5^2+5.9^2)}{((-9.8)-0.7)2}$$

n=13.58

As the allowance for 10%drop outrate,the sample size was set 15 subjects per group ,The total were 30 subjects.

Sample Selection

Patients were randomly selected from those who came to the Prempracha Medical Clinic, Bangkok. Patients were clearly explained on the purpose of the study. They were given a preliminary test using FBG analyzer to discriminate if inclusion criteria were met. Inclusion criteria; (a)Male and female were at the age of 25-50 years old. (b)Patients had FBG in range 100-125 mg/dL. (c)Patients were not diagnosis with diabetes and exclusion criteria;(a)Allergic to Biotin and Brewers' yeast.(b)Patients with known abnormality of renal and liver function.(c)Patients who used medicine acting on blood glucose level such as metformin.(d)Pregnant women. After enrollment, all patients were allocated into two groups; one based on lifestyle modification with Biotin supplement the other based on lifestyle modification with Biotin plus Brewers' Yeast.

Scope of Research

The patients who were circumference in Bangkok, male and female and aged between 25-50 years old, They
came to Prempracha Medical Clinic , Bangkok , during February - June 2015. Their fasting blood glucose level has to be in a range of 100-125 milligram/deciliter. All patients were not diagnosis to be diabetic patients. All patients were divided into two groups; Control group: Receiving Biotin 2400 microgram/day; Experimental group : Receiving Biotin 2400 microgram/day plus

Brewers' Yeast 2000 milligram/day divided 1000 milligram 2 times a day.All patients would be required to come back to Prempracha Medical Clinic,

Bangkok,after 4 weeks , 8 weeks , and 12 weeks of supplementation. Follow up measures included general interview, fasting blood glucose , HbA1c , body weight , height and body mass index , respectively. Data on changes of fasting blood glucose and HbA1c were analyzed and compared within the group at 4 weeks, 8 weeks and 12 weeks and between groups at the same period of investigation.

Research Design

This study was a randomized controlled-trial experimental research to study the efficacy of Biotin plus Brewers' Yeast in lowering fasting blood glucose level compared with Biotin alone in prediabetes.30 patients were divided in two groups; 15 patients /group. The control group received Biotin 2400 microgram/day divided 1200 microgram in the morning and in the evening. The experimental group received Biotin 2400 microgram/day divided1200 microgram in the morning and in the evening plus Brewers' Yeast 2000 milligram/day divided 1000 mg in the morning and in the evening. Then, the efficacy of results measuring from patients' blood glucose and HbA1c levels before the ingestion and every 4 weeks afterward at 4th week, 8th week and 12th week. The study also compared the effectiveness between two groups. Study variables included: Independent variable including the supplement; Biotin and Biotin plus Brewers' Yeast. Dependent variable including the change in fasting blood glucose and HbA1

Research Tools

Research tools included

(a) Biotin (600 microgram/capsule) 2 capsules in the morning and 2 capsules in the evening for 2400 microgram/day,

(b)Brewers' Yeast (500 milligram/tablet) 2 tablets in the morning and 2 tablets in the evening for 2000

milligram/day, (c) FBG, HbA1c and Blood lipid level from collecting venous blood test, (d) Informed consent form, (e) Physical and laboratory examination form, and

(f) Daily diet form

Procedures

Patients were selected according to inclusion and exclusion criteria. Each patient were described objectives, methods and benefit from our research study. Each patient signed the informed consent and reveal their personal history of drug allergy and disease. Each patient were examine and measure fasting blood glucose and HbA1c.All patients were interviewed on general information, life styles, medical history. Investigator keeps record of all date obtained in research record form. Investigator of the experiment randomly assigned each patient into two groups; the control and the experiment group. Investigator explained the proper use of the supplement as follows :Control group intake two capsules of Biotin (600 microgram/capsule) after meal in the morning and two capsules after meal in the evening (total 2400microgram/day*)* (Albarracin *etal.*,2007). Experimental group intake two capsules of Biotin (600 microgram/capsule) after meal in the morning and two capsules after meal in the evening (total 2400 microgram/day*)* combined with two tablets of Brewers' Yeast (500 milligram/tablet) after meal in the morning and two tablets after meal in the evening (total 2000milligram/day) (Ali *et al.*,2011). The benefit and possible side effects were explained to each patient. The supplements were given enough amount for the period of weeks. Patients were then required to come back for follow up. Patients were given follow up FBG by the end of 4th and 8th weeks. Their nutritional lifestyle and behavior during an experiment were also interviewed. Patients came back at the end of the 12th week for follow up. FBG, HbA1c and Blood lipid level were re-determined.

Data Collection

Investigator performed data collection upon each follow-up visit by measuring the value of FBG, HbA1c and blood lipid level. Investigator recorded patients' body weight, height, waist circumference, Body mass index at 4th week, 8th week and 12thweek, respectively.

Statistical Analysis

Descriptive statistics were used to demonstrate demographic data of patients. Comparing the mean of changes of fasting blood glucose between control and experiment groups by t-test 95% level of confidence, p value ≤ 0.05. Comparing the mean of change HbA1c between control and experiment groups by t-test95% level of confidence, p value ≤ 0.05. Comparing the mean offasting blood glucose at beginning, 4th weeks, 8th week and 12th week in each group by two -way ANOVA Repeated Measurement95% level of confidence, p value ≤ 0.05. Comparing the mean of HbA1c at beginning and 12th week in each group by paired t-test.95% level of confidence, p value ≤ 0.05.

Results

Among 30 enrolled participants, 27 subjects completed the study.

The general characteristics of Samples

Table 1 The general characteristic of the control group (Biotin) and the experimental group (Biotin plus Brewers' yeast)

	Biotin (n=13)		Biotin plus Brewers' Yeast (n=14)		
	Mean	**SD**	**Mean**	**SD**	**P-value**
Age (year)	39.69	8.78	38.21	8.30	0.66
Sex (%)					
Male	7 (53.8%)		7 (50.0%)		0.84
Female	6 (46.2%)		7 (50.0%)		
Body weight (kg.)	57.85	5.70	55.07	8.08	0.32
BMI(kg/ ²)	22.31	1.39	21.53	2.25	0.29
FBS (mg/dl)	113.08	7.94	111.64	7.30	0.63
HbA1C (%)	5.23	0.73	5.00	0.78	0.44
Total cholesterol (mg/dl)	199.85	25.78	200.07	21.20	0.98
LDL cholesterol (mg/dl)	123.25	20.13	128.57	21.92	0.53
HDL cholesterol (mg/dl)	50.77	7.96	55.14	6.13	0.12
Triglyceride (mg/dl)	132.23	28.22	117.57	22.63	0.15
Systolic blood pressure (mm/Hg)	124.77	9.62	121.43	9.28	0.37
Diastolic blood pressure (mm/Hg)	76.92	8.50	74.14	6.43	0.34

According to Table 1, most of the sample subjects were female about50.0% in the experimental group and 46.2% in the control group. The average age of both groups were the same level in which 38.21± 8.30 years old in the experimental group and39.69 ± 8.78 years old in the control group. Similar to other general characteristics, body weight , body mass index, FBG level, HbA1C, Total cholesterol, LDL cholesterol, HDL cholesterol, Triglyceride, Systolic blood pressure an d Diastolic blood pressure at the starting point had no statically different between two groups.

Table 2 Number of samples with normal fasting blood glucose (FBG 70-100 mg/dL)

Periods	FBG in normal range			
	Biotin		Biotin plus Brewers' Yeast	
	n	**Percentage**	**n**	**Percentage**
Beginning	0	0	0	0
4th week	0	0	3	20
8th week	2	13.4	1	6.7
12th week	1	6.7	4	26.66

According to Table2,the experimental group had fasting blood glucose decreasing to normal range about 20% at the 4thweek,6.7% at 8th week and 26.66% at 12th week, respectively. The control group had some subjects who had fasting blood glucose decreasing in the normal range about 13.4 % at the 8th week and 6.7% at 12th week, respectively.

Comparative analysis of body weight

Table 3 Comparative analysis of body weight between the control group (Biotin) and the experimental group (Biotin plus Brewers' Yeast)

	Biotin (n=13)		Biotin plus Brewers' Yeast(n=14)		
	Mean	**SD**	**Mean**	**SD**	**P-value**
Body weight					
Beginning	57.85	5.70	55.07	8.08	0.09
4thweek	58.00	6.48	53.83	6.63	0.13
8th week	57.77	6.44	55.14	7.35	0.14
12th week	57.92	6.53	55.36	7.94	0.14
p-value within group					
Beginning vs. 4thweek	1.00		1.00		
Beginning vs. 8thweek	1.00		1.00		
Beginning vs. 12thweek	1.00		0.93		
Difference; Beginning - 4thweek	0.15	1.21	0.33	1.23	0.72
Beginning - 8th week	-0.08	1.19	0.07	1.27	0.76
Beginning - 12thweek	0.08	1.19	0.29	0.73	0.5

According to table 3, the body weight of these two groups had rather stable in all four periods of times. When comparing the average body weight in each period between these two groups found no statistically difference at p-value =0.09, 0.13,0.14 and0.14, respectively. When comparing the body weight in the same group of each period showed that the experimental group had rather stable body weight by p = 0.100 as measuring in all three periods when compared to the body weight at the starting point. Similar to the control group whose body weight had rather stable at the 4th, 8th and 12th weeks at p = 1.00, 1.00 and 0.93, respectively. However , when investigating the changes of body weight , it showed that in the 4th , 8th , and 12th week , the body weight changed of these two groups were not statistically difference from each other as p= 0.72,0.76, and 0.59 , respectively

.

Comparative Analysis of Body Mass Index (BMI)

Table 4 Comparative analysis of Body Mass Index (BMI) between the control group (Biotin) and the

experimental group (Biotin plus Brewers' yeast)

	Biotin (n=13)		Biotin plus Brewers' Yeast(n=14)		
	Mean	**SD**	**Mean**	**SD**	**p-value**
Beginning	22.31	1.39	21.53	2.25	0.10
4th week	22.31	1.68	21.20	2.25	0.17
8th week	22.23	1.69	21.47	2.09	0.18
12th week	22.28	1.73	21.60	2.24	0.19
p-value within group					
Beginning vs. 4th week	1.00	1.00			
Beginning vs. 8th week	1.00	1.00			
Beginning vs. 12th week	1.00	1.00			
Difference; Beginning - 4th week	0.00	0.50	0.10	0.48	0.62
Beginning - 8th week	-0.08	0.44	-0.05	0.57	0.89
Beginning - 12th week	-0.03	0.47	0.08	0.31	0.50

According to table 4, it shows that both groups had rather stable body mass index in all periods of times. When comparing body mass index between the control group and the experimental group, there was no statistically difference from each period p=0.10, 0.17, 0.18, and 0.19, respectively. However,when comparing body mass index in the same group with differentperiods, it shows that the experimental group and the control group had rather stable body mass index by p = 1.0 in all three times of measurement.When comparing these two groups between the beginning of eachperiod at the 4th,the 8th and the 12th weeks found that there were no statistically differences. However ,the changes of body mass index change showed that both of these groups at the 4th, the 8th and the 12th weeks had no statistically difference byp=0.62,0.89 and 0.50 , respectively.

Comparative Analysis of Blood Glucose level

Table 5: Comparative analysis of blood glucose level between the control groups (Biotin) and the experimental group (Biotin plus Brewers' yeast)

	Biotin (n=13)		Biotin plus Brewers' Yeast(n=14)		
	Mean	**SD**	**Mean**	**SD**	**P-value**
FBG(mg/dl)					
Beginning	113.08	7.94	111.64	7.30	0.85
4th week	105.85	15.72	100.17	20.13	0.44
8th week	103.3	19.55	102.43	15.13	0.58
12th week	104.46	16.81	103.43	16.67	0.66
p-value within group					
Beginning vs. 4th week	0.45		0.03*		
Beginning vs. 8th week	0.02*		<0.001 **		
Beginning vs. 12th week	0.09		0.03*		
Difference; Beginning - 4th week	-7.23	11.13	-12.33	16.49	0.37
Difference;Beginning - 8th week	-9.77	7.53	-9.21	13.40	0.90

	Biotin (n=13)		Biotin plus Brewers' Yeast(n=14)		
	Mean	**SD**	**Mean**	**SD**	**P-value**
FBG(mg/dl)					
Difference; Beginning - 12th week	-8.62	12.45	-8.21	13.62	0.94
Percentage of changes in 12th week	-7.89	11.35	-7.53	12.42	0.94
HbAC (%)					
Beginning	5.23	0.73	5.00	0.78	0.44
12th week	5.02	0.61	4.94	0.75	0.77
p-value within group	0.20		0.69		
Difference ; Beginning - 12th week	-0.23	0.54	-0.06	0.65	0.48
Percentage of changes in 12th week	-3.64	10.95	-0.42	12.89	0.50

Note*p < 0.05 , ** p < 0.001

According to table 5, the FBG level in both groups, tended to be decreased in all four periods. When comparing the average level of FBG in each period between both groups, there were no statistically difference in all four period as p= 0.81, 0.44, 0.58, and 0.66, respectively. When considering duration of time found that duration of time taking medication effected FBG level (p <0.001) by comparing the FBG level in the same group. It also showed that the experimental group had FBG level decreasing significantly since the 4th weeks as p=0.03,<0.001, and0.03, respectively. On the other hand , the control group showed that the FBG level decreased only in the 8th weeks at p =0.02 , whereas in the 4th, 8th and 12th weeks , the average level of FBG changes in both groups had no statistically difference at p=0.37, 0.90 , and 0.94, respectively. HbA1c were measured at the beginning and the 12th weeks illustrated that both the experimental and the control group tended to be decreased of the HbA1C level. When comparing the average of HbA1C level in each period between these two groups, the difference was no statistically significant at p=0.44 and 0.77, respectively. When comparing the HbA1C level in the same group showed that both groups had decreasing the HbA1C level but without statistically significant. The HbA1C change showed no significant difference in average HbA1C level between these two groups at p=0.48. (Figure1 –Figure 4)

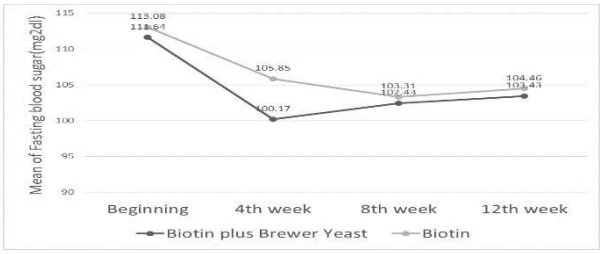


Figure 1: Comparison of Fasting blood glucose level at the beginning, 4th, 8th and 12thweeks, respectively, between the control group (Biotin) and the experimental group (Biotin plus Brewers' Yeast)

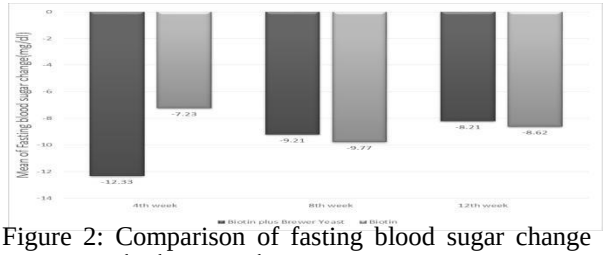


Figure 2: Comparison of fasting blood sugar change from the 4th,8th and 12th weeks, respectively, between the control group (Biotin) and the experimental group (Biotin plus Brewers' Yeast)

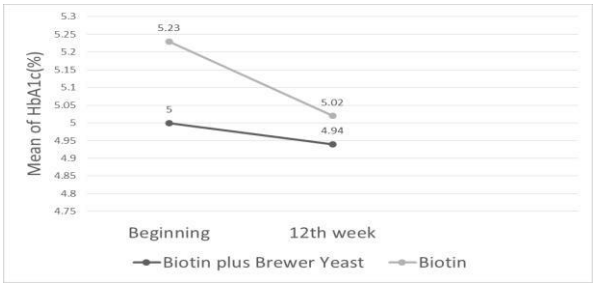


Figure 3: Comparison of HbA1C level at the beginning and 12th weeks between the control group (Biotin) and the experimental group (Biotin plus Brewers' Yeast)

Figure 4: Comparison of HbA1C change from the beginning and 12th weeks between the control group (Biotin) and the experimental group (Biotin plus Brewers' Yeast)

Comparative Analysis of Blood Pressure Level

Table 6 Comparative analysis of blood pressure level between the control group(Biotin) and the experimental group (Biotin plus Brewers' Yeast)

	Biotin (n=13)		**Biotin plus Brewers' Yeast(n=14)**		
	Mean	**SD**	**Mean**	**SD**	**P-value**
Systolic blood pressure (mmHg)					
Beginning	124.77	9.62	121.43	9.28	0.31
4th week	122.38	9.60	121.92	8.44	0.90
8th week	124.31	6.28	122.14	7.62	0.35
12thweek	121.77	7.55	121.21	7.77	0.66
p-value within group					
Beginning vs. 4th week	1.00		1.00		
Beginning vs. 8th week	1.00		1.00		

	Biotin (n=13)		**Biotin plus Brewers' Yeast(n=14)**		
	Mean	**SD**	**Mean**	**SD**	**P-value**
Diastolic blood pressure (mmHg)					
Beginning	76.92	8.50	74.14	6.43	0.25
4th week	78.08	6.98	68.50	14.34	0.04*
8th week	81.23	5.45	71.21	7.26	<0.001**
12th week	78.38	5.72	74.36	7.70	0.21
p-value within group					
Beginning vs. 4th week	1.00		0.98		
Beginning vs. 8th week	0.84		1.00		
Beginning vs. 12thweek	1.00		1.00		

Note*p < 0.05 , ** p < 0.001

According to table 6 , it was found that the systolic blood pressure in these two groups were rather stable in all four periods and no statistical difference when comparing the mean blood pressure in both group at p=0.31,0.90,0.35 , and 0.66 , respectively. While considering about the duration of time, it showed that the time was not effect to the systolic blood pressure change statistically (p= 0.66). When analysis the systolic blood pressure in each period of time in the same group, it showed that the systolic blood pressure rather stable by p=1.00 in all three periods from the beginning except in the 12th weeks of the control group (p =0.80).For the diastolic blood pressure ,it showed that the experimental group tended to decreasing of diastolic blood pressure while increasing in the control group .When comparing the mean diastolic blood pressure in each periods between these two groups , it should that they were statistically difference in the measurement of 4thweek and the 8th week at p=0.04, and <0.01, respectively. When considering the time of experiment, it showed that no statistically effect to the diastolic blood pressure (p=0.41) by diastolic blood pressure at each period in the same group rather stable (the

experimental group: p=1.00, 0.84, and 1.0; the control group: P value =0.98, 1.00 and1.00).

Comparative Analysis of Blood Lipid Level

Table7: The comparative analysis of blood lipid level between the control group (Biotin) and the experimental group (Biotin plus Brewers' Yeast)

	Biotin (n=13)		Biotin plus Brewers' Yeast(n=14)		
	Mean	**SD**	**Mean**	**SD**	**P-value**
Total cholesterol (mg/dl)					
Beginning	199.85	25.78	200.07	21.20	0.98
12th week	198.92	18.62	196.71	28.57	0.82
Difference ; Beginning - 12th week					
	-0.92	22.75	-3.36	16.69	0.7
LDL cholesterol (mg/dl)					
Beginning	123.25	20.13	128.57	21.92	0.53
12th week	131.62	19.89	121.00	21.60	0.3
p-value within group		0.13		0.04*	

Biotin (n=13)			**Biotin plus Brewers' Yeast(n=14)**		
Mean	**SD**		**Mean**	**SD**	**P-value**
Difference; Beginning - 12th week					
5.92	12.82		-7.57	13.00	0.01*
Percentage of changes in 12th week					
5.61	10.10		-5.62	10.12	0.01*
HDL cholesterol (mg/dl)					
Beginning	50.77	7.96	55.14	6.13	0.12
12th week	52.92	7.80	56.43	5.46	0.19
Difference; Beginning - 12th week					
2.15	4.41		1.29	4.43	0.61
Triglyceride (mg/dl)					
Beginning	132.23	28.22	117.57	22.63	0.15
12th week	127.00	21.22	119.57	24.26	0.41
Difference ; Beginning -12th week					
-5.23	32.37		2.00	16.93	0.47

According to table 7, it shows that there was no statistical difference of the level change of total cholesterol, HDL cholesterol and triglyceride to the duration of medical intake. On the other hand, it found that LDL cholesterol decreased in the experimental group while increased from the beginning in the control group. When comparing the average LDL cholesterol level in each measuring between these two groups, it showed no statistical difference at p=0.53and 0.32,respectively.When considering about the duration of medical taking found that it effected to the LDL cholesterol level. The experimental decreased significantly at p=0.04, while there was the increase in the control group but without statistical significant by p= 0.13. Analysis of LDL cholesterol change also illustrated that the average of LDL cholesterol change in both groups at the 12th weeks were different implied that the average decreased at 7.57± 13.00mg/dl in the experimental group and the average increased at 5.92 ±12.82 mg/dl in the control group ,with statistical difference at p=0.01.(Figure 5-Figure 10).

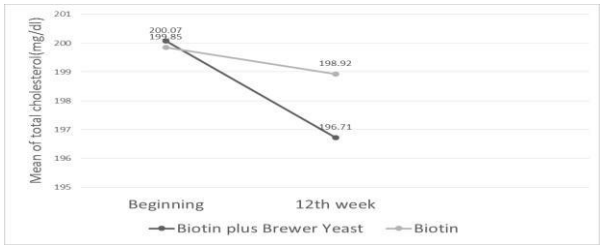


Figure 5: Comparison of total cholesterol at the beginning and 12th weeks between the control group (Biotin) and the experimental group (Biotin plus Brewers' Yeast)

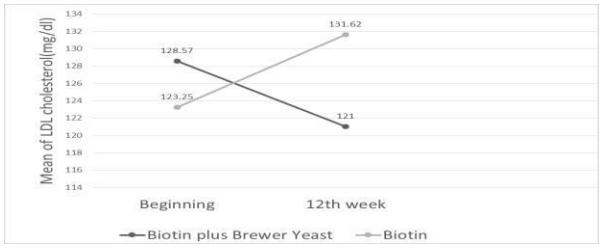


Figure6: Comparison of LDL cholesterol at the beginning and the 12th weeks between the control group (Biotin) and the experimental group (Biotin plus Brewers' Yeast)

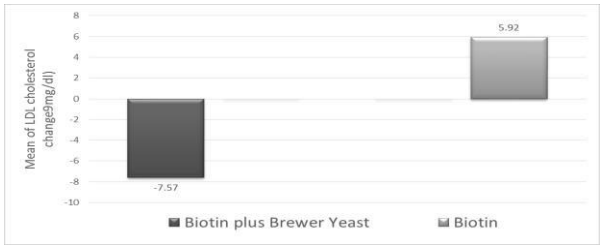


Figure7: Comparison of LDL cholesterol change from the beginning and 12thweeks between the control group (Biotin) and the experimental group (Biotin plus Brewers' Yeast)

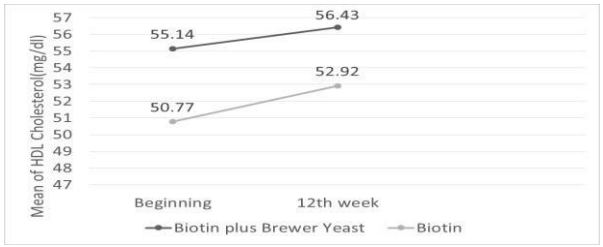


Figure 8: Comparison of HDL cholesterol at the beginning and 12th weeks between the control group (Biotin) and the experimental group (Biotin plus Brewers' Yeast)

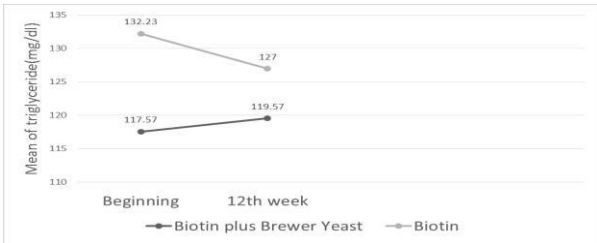


Figure 9: Comparison of Triglyceride at the beginning and 12th weeks between the control group (Biotin) and the experimental group (Biotin plus Brewers' Yeast)

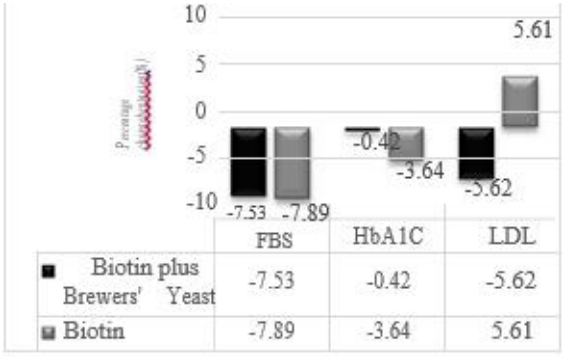


Figure 10: Comparison average percent of changing from the beginning and 12th weeks of FBS, HbA1C, LDL cholesterol between the control group (Biotin) and the experimental group (Biotin plus Brewers' Yeast)

Discussion

From the previous study :Combination of Chromium and Biotin Supplementation in Glycemic Control in Prediabetes (Danaei,1980) The total of 30 prediabetes were enrolled to receive either 800 microgram of chromium picolinate and 2 milligram of biotin, or matching placebo, for 5 weeks. Major endpoints were reductions in fasting plasma glucose, serum triglyceride and increase in HDL cholesterol, serum creatinine. The study showed that the combination of chromium and biotin supplementation did not have the efficacy in glycemic control in prediabetes over the chromium supplementation alone. But in this study found that the experimental group and the control group can lowering blood glucose in prediabetes .Considering the period of study is may effect to blood glucose level because the previous research study in 5 weeks but in the author's research study in 12 week.

Chromium picolinate and biotin combination improves glucose metabolism in treated, uncontrolled overweight to obese patients with type 2 diabetes . The study had four hundred and forty-seven subjects with poorly controlled type 2 diabetes (HbA1C ≥ 7.0 %) . They were enrolled and received either chromium picolinate (600 microgram) with biotin (2 milligram), or matching placebo, for 90 days in combination with stable oral anti-diabetic agents (OADs). Major endpoints were reductions in HbA1C, fasting glucose, and lipids. The study founded HbA1C in the chromium picolinate/biotin group decreased 0.54%. The decrease in HbA1C was most pronounced in chromium picolinate/biotin subjects whose baseline HbA1C > or = 10%, and highly significant when compared with placebo (-1.76% vs. - 0.68%; p = 0.005). Fasting glucose levels were reduced in the entire chromium picolinate/biotin group versus placebo (-9.8 mg/dL vs 0.7 mg/dL; p = 0.02). Reductions in fasting glucose were also most marked in those subjects whose baseline HbA1C > or = 10.0%, and significant when compared to placebo (-35.8 mg/dL vs. 16.2 mg/dL; p = 0.01).These results suggest that the chromium picolinate with biotin, administered as an adjuvant to current prescription anti-diabetic medication, can improve glycaemic control in overweight to obese individuals with type 2 diabetes; especially those patients with poor glycaemic control on oral therapy (Albarracin, 2007) The author's study show Biotin and Brewers' Yeast can lowering blood glucose in Prediabetes but the subject in the previous study were diabetic patients so in the author study used Biotin low dose than the previous. Dosage of supplements may involve efficacy of supplement.

Chromium effects on glucose tolerance and insulin sensitivity in persons at risk for diabetes mellitus .This study designed by Ali*et al.*(2011) to investigate the effects of daily chromium picolinate supplementation on serum measures of glucose tolerance and insulin sensitivity in patients at high risk for type 2 diabetes mellitus. Adult patients with impaired fasting glucose, impaired glucose tolerance, or metabolic syndrome were enrolled. Participants received 6 month sequences of chromium picolinate or placebo at 1 of 2 dosages (500 or 1000 microgram daily). After 6 months of chromium at either dosage level (500 microgram or 1000 microgram daily) when compared with placebo. None of the secondary outcomes improved with either chromium dosage compared with placebo (P value > 0.05). From this study, The Chromium supplementation does not appear to ameliorate insulin resistance or impaired glucose metabolism in patients at risk for type 2 diabetes and thus is unlikely to attenuate diabetes risk(Ali*et al.,* 2011). In the author study show Biotin and Brewers' Yeast can lowering blood glucose in Prediabetes in 12th week but studied in 6 months in this previous study .If considering of period to study, long period it more difficult to control variable than short period.

Conclusion

This study aimed to compare the fasting blood

glucose-lowering effects of the combination of Biotin and Brewer yeast versus Biotin alone in prediabetes. The comparative Fasting blood glucose-lowering effects of the experimental group (Biotin plus Brewer Yeast) versus the control group (Biotin alone) had tendency of fasting blood glucose level to be decreased in 4 times measuring by p = 0 . 8 1 , 0 . 4 4 , 0 . 5 8 and 0 . 6 6 ,respectively. Duration of time taking Biotin and Brewer Yeast had effect on FBS.The experimental group had FBS level decreasing significantly since 4th weeks by p = 0.03. About the control group found that the FBS level decreased only in the 8th weeks by p = 0.02.When considering LDL cholesterol, in the experimental group decrease significantly at p = 0.04 while increase in control group but without statistic significant by p = 0.13.Both groups can reduce fasting blood glucose level in prediabetes but the experimental group (Biotin plus Brewer Yeast) can reduce fasting blood glucose faster than the control group (Biotin alone) significantly.This study shown that Biotin and Brewers Yeast are natural treatments for lowering blood glucose in prediabetes.Then there can reduce complication from type 2 diabetes and side effect of antidiabetic medication.Because of limited time to study the author could not finding side effect of Biotin and Brewers' Yeast on blood glucose level in this study.If the author has more time to study,the author will find the significantly difference between two groups.

References

Albarracin, C., Fuqua, B., Geohas, J., Juturu, V., Finch, M. R. and Komorowski, J. R. (2007). Combination of chromium and biotin improves coronary risk factors in hypercholesterolemic type 2 diabetes mellitus: A placebo-controlled, double-blind randomized clinical trial. *Journal of the Cardiometabolic Syndrome*, *2*(2), 91-97.Ali,A., Ma,Y., Reynolds,J. Wise, J., Inzucchi,S.E. andKatz,D.L. (2011). Chromium effects on glucose tolerance and insulin sensitivity in persons at risk for diabetes mellitus. *Endocr Prac,17*(1),16-25.

Danaei,G. and Lu, F.Y. (1980). *National, regional,and global trends in fastingplasma glucose and diabetes prevalence since 1980*.Boston: Global public.

Global status report on noncommunicable diseases 2010 (2011). Geneva: World Health Organization.

Roglic,G., Unwin, N., Bennett, PH., Mathers,C., Tuomilehto,J. and Nag, S. (2005). The burden of mortality attributable to diabetes: realistic estimates for the year 2000. *Diabetes Care*, 28(9), 2130–2135.

Mathers, C.D. and Loncar, D. (2006).Projections of global mortality and burden of disease from 2002 to 2030. *PLoS Med*, *3*(11), e442.

World Health Organization.(2014). *Deaths by Cause, Age, Sex and Country, 2000-012*. Geneva:WHO.

World Health Organization.(2012). *Global status reporton noncommunicable diseases 2014*. Geneva: WHO.

World Health Organization (1999).*Definition, diagnosisand classification of diabetes mellitus and its complications, 1999, Part 1: Diagnosis and classification of diabetes mellitus*. Geneva:WHO.

The Efficacy and Safety of 5% tTpical Minoxidil Versus 3% Topical Minoxidil for Beard Enhancement: A Randomized, Double-blind, Comparative Study

Chinnawat Reanmanee and Chuchai Tanglertsampan

School of Anti-Aging and Regenerative Medicine, Mae Fah Luang University, Bangkok, Thailand
**Corresponding author. E-mail:drchuchait@yahoo.com*

Abstract

At present, patients who need to enhance their beard to build up their confidence and personality applying topical minoxidil lotion which was used as off-label. Scantly report studying the efficacy of minoxidil lotion in stimulating hair growth at chin. We demonstated in the previous study that 3% minoxidil lotion was significantly superior to placebo in beard enhancement. **Objective:** To compare the efficacy and safety of 5% minoxidil lotion and 3% minoxidil lotion for enhancing beard hair in Thai men. **Materials and Methods:** 52 male participants , aged between 20-60 years, were randomly applied 5% minoxidil lotion and 3% minoxidil lotion. daily for 16 weeks and follow-up every 4 weeks. The result was evaluated by using global photographic score, beard hair diameter, side effects, and paticipants' satisfaction. **Results:** 47 of 52 participants completed the study. After 16 weeks, participants receiving 5% minoxidil had higher global photographic score but not statistically significant($p>0.05$) compared to those whom applied 3% minoxidil lotion. When considering secondary outcome (beard hair count), 5% minoxidil lotion showed significantly superior to 3% minoxidil lotion for beard hair count at week 8^{th} 12^{th} 16^{th}. However, 5% minoxidil lotion showed no statistic difference in hair diameter and participants' satisfaction compared to 3% minoxidil lotion. Pruritus and tingling were the side effect recognized in both groups. **Conclusion:** Although 5% minoxidil lotion showed trend of more efficacy than 3% minoxidil lotion, it was not statistically significant. Both concentration are safe for topical treatment with only mild side effect. With the plethora of anti-hairloss formulas for beard hair stimulation, most of which have not been tested in randomized, double-blinded trials. So well-controlled trials that demonstrated the efficacy and safety of topical remedies is indispensable.

Keywords: Minoxidil, Lotion, Topical, Beard enhancemen

Introduction

Human body is comprised of fine light-colored hair and thick long dark hair especially in chin area. After puberty, hairs in this area turn from vellus hair to terminal hair under influence of male hormone, androgen transforming less visible, fine hair into more noticeable, dark hair which indicates adult male's appearance. In prehistoric era, ancient male grew their beard on purpose of keeping their body warm, intimating their enemy. Apart from physical importance, beard hair plays role in other aspects. For example, in evolutionary view, Darwin pointed out that beard hair was one of the considerations in mating preference. In psychologic and social behavior aspect, bearded men are perceived as trustworthy, kindness and male predominance. So the trend of growing bread is increasing.

Some diseases such as alopecia areata, hypothyroidism, trauma, certain malignancies, infections can cause losing hairs effecting patients' confidence, as a result, patients seek medical treatment for growing their hair back.

Recent studies show that hair transplantation at mandibular area is so effective however the cost of this procedure is quite high and the risk is post-operative infection. Topical minoxidil is other options approving by FDA in treatment of AGA. Using topical minoxidil for beard hair growth is off-label use. Previous study revealed that 3% minoxidil showed effectiveness in stimulating beard hair growth compared to placebo.

Materials and Methods

Objective

To compare efficacy and safety of 5%minoxidil lotion and 3% minoxidil lotion in stimulating beard hair growth

Research methodology

Patient population

Men eligible for inclusion in the study were 20 to 60 years old and in good general health with no evidence of systemic illnesses (eg, cardiac, renal, hepatic disease). They needed to enhance their beard. Men who were suffered from skin disease (eg, atopic dermatitis, keratosis pilaris atropicans, alopecia areata, DLE, cutaneous T-cell lymphoma) and systemic diseases causing hair loss (eg, cushing syndrome, hyper- or hypothyroidism) were excluded, as were patients known to be hypersensitive to minoxidil solution or who concomitantly used hair restorers or systemic drugs (steroids, cytotoxic agents, vasodilators, antihypertensives, anticonvulsant drugs, beta-blockers, diuretics, or any of the following specific agents: spironolactone, cimetidine, diazoxide, cyclosporine, ketoconazole, or replacement hormonal therapy).

Study design

This was a 16-week, randomized, double-blinded, clinical trial conducted in Thailand between November 2015 to Febuary 2016. The protocol and informed consent form were approved by The Mae Fah Luang University Research Ethics Committee on Human Research, and written informed consent was obtained from each volunteer before enrollment in the trial. 5% minoxidil lotion or 3% minoxidil lotion were randomly given using block randomization. Volunteers were instructed to apply minoxidil lotion twice daily for 16 weeks then were evaluated at 4th , 8th 12th and 16th weeks. Global photographic score measured by VISIA® Complexion Analysis System before treatment and at 4th , 8th 12th and 16th weeks were compared and scored by 3 dermatologists. . Hair diameters and hair count were measured by Folliscope. Volunteers' side effects were assessed by questionnaires and physician's observation. Volunteers' satisfaction was evaluated at 16th week by questionnaires.

Statistical Analysis

SPSS (Chicago, IL,USA) was used for the statistical analysis.Global photographic score were differentiated The Mann-Whitney U test.Evaluating difference of beard hair diameter and beard hair count by independence samples t-test. Evaluating side-effects by Fisher-Exact test. Volunteers' satisfaction were assessed using The Mann-Whitney U test. The researcher did the following at the confidential level of 95% and the significance levels of p-value <0.005.

Result

Out of 52 cases, 47 completed the study. Global photographic score of 5% minoxidil lotion and 3% minoxidil lotion demonstrated statistical significance at 12th week even though ,at 16th week, there is no statistic significance (p-value = 0.033 and 0.312 , respectively)(figure 1). There was statistically insignificant in difference of beard hair diameter between 5% minoxidil and 3% minoxidil (figure2). However, paired differences of beard hair count showed statistic significance in results at 8th 12th and 16th(p-value<0.05)(figure3). No statistically significant difference of side effects and volunteer's satisfaction between application of 5%minoxidil lotion and 3% minoxidil lotion. The most common side effects of minoxidil application were pruritus and tingling sensation.

Figure1: Comparion of Globalphotographic score between 5% minoxidil lotion and 3% minoxidil lotion

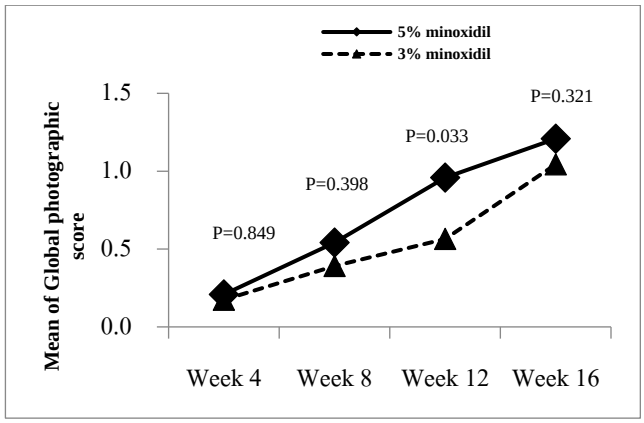


Figure2 :Comparing of difference of the mean of beard hair diameter between 5% minoxidil lotion and 3% minoxidil lotion

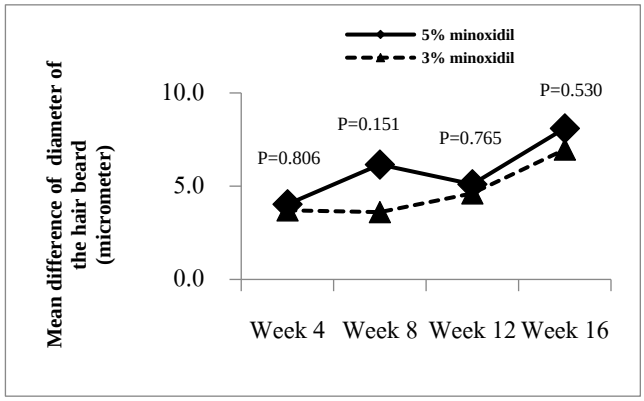
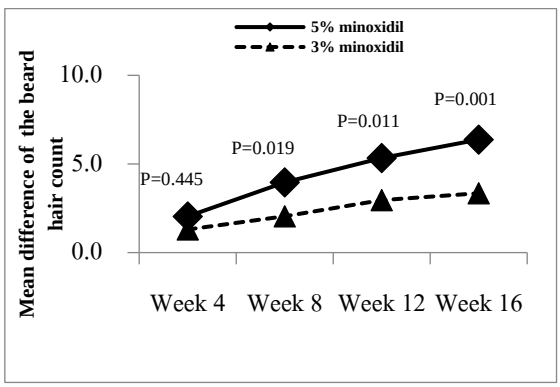
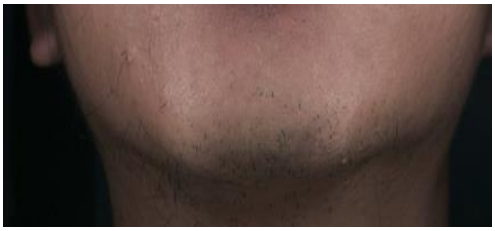


Figure3: Comparng difference of the mean of beard hair count between 5% minoxidil lotion and 3% minoxidil lotion

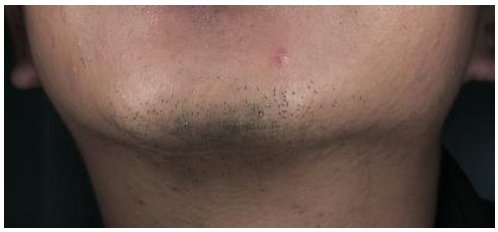


5% Minoxidil lotion

Before treatment

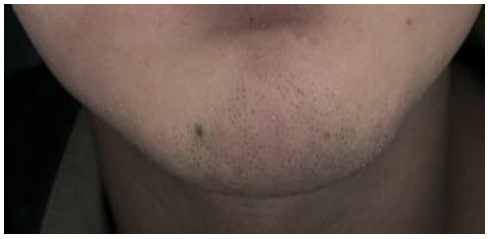


After 16th week treatment

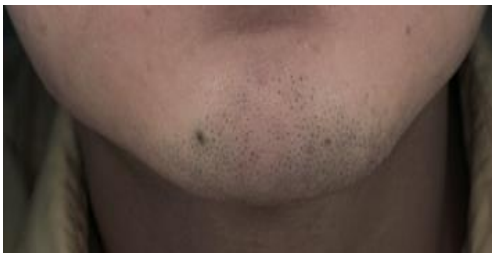


3% Minoxidil lotion

Before treatment



After 16th week treatment



Discussion

Topical minoxidil can stimulate the growth and development of human hair which correlated with the study of Olsen et al, 2002; Lee et al, 2014; and Sittichai I, 2016. From this study we found that 5% minoxidil lotion showed superior efficacy to 3%minoxidil by considering global photographic score at 1 2 th week however, at 16th week, there was no statistically significant between 2 concentation. In addition, 5% minoxidil is statistically significant effective in increasing number of beard hair over 3% minoxidil lotion. The finding of increased beard hair counts over the course of the 16-week trial provides evidence that topical minoxidil not only reverse beard hair loss but also slow the progression of beard hair loss.

This study is consistent to the previous study found that there was no statistic significant in the difference of beard hair diameter. Not only Volunteers' satisfaction, but recorded side effects among two groups also appeared statistical insignificant too. Drug-related adverse events of dermatology (eg, pruritus, dermatitis, hypertrichosis, scaling) were the same prevalent between two groups. All adverse events were mild and it can be disappeared despite continued use of the drug

The mechanism by which topical minoxidil induced beard hair growth has not been fully characterized. Topical minoxidil is postulated to increase beard hair density either by induction of anagen or anincrease in anagen duration. Hair diameter is also increased by topical minoxidil. The net result is reversible of the miniaturization process, slowing the progression of beard hair loss, or both

Conclusion

Although 5%minoxidil lotion showed trend of more efficacy than 3%minoxidil lotion, it was not statistically significant. Both concentrations are safe for topical treatment with only mild side effect. With the plethora of herbal formulas for beard hair stimulation, most of which have not been tested in randomized, double-blinded trials. So well-controlled trials that demonstrated the efficacy and safety of topical remedies are indispensable.

Suggestion

We suggest further study to lengthening the duration of treatment on 5% minoxidil lotion and 3% minoxidil lotion in order to demonstrate the maximum effect of these drugs and withdrawal effect of the drug after stop applying these solutions.

Reference

1. Inui S, Itami S. Androgen actions on the human hair follicle perspectives. Exp Dermatol 2013;22(3):168-71
2. Fink B, Nick N. The biology of facial beauty. International Journal of Cosmetic Science, 27 (2005), pp. 317–325
3. Nick N, Kerry S. The effects of facial hair manipulation on female perceptions of attractiveness, masculinity, and dominance in male faces. Personality and Individual Differences Volume 45, Issue 5, October 2008, p. 373–377
4. Alkhalifah, A. Alopecia areata update. Dermatol Clin,2013;31(1), 93-108.
5. Van Beek N, Bodo E, Kromminga, A, Gaspar E, Meyer K. Thyroid hormones directly alter human hair follicle functions: anagen prolongation and

stimulation of both hair matrix keratinocyte proliferation and hair pigmentation. J Clin Endocrinol Metab,2008;93(11), 4381-4388.
6. Yeager CE, Olsen EA, Treatment of chemotherapy-induced alopecia. Dermatol Ther,2011;24(4), 432-442.
7. Wall D, Fraher M. Infection of the Beard area. Kerion: a review of 2 cases.Ir Med J. 2014 Jul-Aug;107(7):219-21.
8. Levesque AY, Schalet BJ, Dolph JL. Beard reconstruction using hair grafts in a free flap. Can J Plast Surg,2011;19(4), 151-152.
9. Konior, RJ. Complications in hair-restoration surgery. Facial Plast Surg Clin North Am,2013;21(3), 505-520.
10. Olsen EA, Dunlap FE, Funicella T, Koperski JA, Swinehart JM, Tschen EH. A randomized clinical trial of 5% topical minoxidil versus 2% minoxidil and placebo in the treatment of androgenetic alopecia in men. J Am Acad Dermatol, 2002;47, 377-385.
11. Lee S, Tanglertsampan C, Worapunpong N. Minoxidil 2% lotion for eyebrow enhancement: a randomized, double-blind, placebocontrolled, spilt-face comparative study. J Dermatol,2014; 41(2), 149-152.
12. Sittichai I, Tanglertsampan C, Tangphianphan N. Efficacy and safety of 3% minoxidil lotion for beard enhancement: A randomized, double-blind, placebo-controlled study. The journal of dermatology, 2016.

The Efficacy of Fractional Insulated Microneedle Bipolar Radiofrequency for Skin Tightening

Pimsawat Watcharakarn[1] and Paisal Rammaneethorn[2]

[1]*Dermatology Program Master of Science Degrees, School of Anti-Aging and Regenerative Medicine, Mae Fah Luang University, Bangkok, 10110, Thailand*
[2]*Corresponding author, School of Anti-Aging and Regenerative Medicine, Mae Fah Luang University, Bangkok, 10110, Thailand*
E-mail : paisalrr@yahool.com

Abstract

Background: Skin laxity is a chief complaint of patients, because it is an aging sign. Radiofrequency, infrared light and HIFU are widely used for treatment of skin laxity, but the efficacy of non-ablative treatments is less than ablative device. **Objective:** To study about the clinical efficacy and other effect of the fractional radiofrequency microneedle device on acquired facial skin laxity in Thai people. **Material and Methods:** Twenty-five participants with facial laxity problem age between 25 – 55 years old were enrolled then treated with fractional insulated microneedle bipolar radiofrequency at week 0, 4th and 8th and patients were followed up at week 4th, 8th and 12th. Clinical outcomes were evaluated by compare a photo, which took by VISIA, between before and after each treatment by 3 independent doctor and also volunteer, skin elasticity was evaluated by Cutometer MPA580 between before and after each treatment and volunteer's satisfaction was evaluated by questionnaire after treatment. Adverse events and patient's satisfaction were recorded. **Results:** Twenty-five Thai patients were enrolled. Four patients was drop out at second follow up (4th week), and twenty-one were evaluated. The subjects were female (21/21, 100%). The average participant age was 33.71 ± 5.98 years old. There were significant differences of mean GAIS since 1 day after treatment. Within first 7 days after treatment. There were statistical significance ($p<0.05$) had increase passive behavior of the skin to force (firmness) on FRM treatment at 8th week ($p<0.05$) and 12th week ($p<0.05$). There were statistical significance ($p<0.05$) had increase ability of returning (gross elasticity) at 12th week ($p<0.05$). **Conclusion:** FRM treatment can be effective treatments for skin tightening. The common side effects are erythema, swelling, post-inflammatory hyperpigmentation and acne occurrence. Pain feeling with and bleeding were indicated immediately after treatment.

Keywords: Skin laxity/Skin tightening/fractional radiofrequency microneedle

Introduction

Radiofrequency, infrared light and HIFU are widely used for treatment of skin laxity (Hyoun S.L. et al, 2012). The use of non-ablative tissue tightening is based on the concept of delivery volumetric heating of the deep dermis and lead to tissue coagulation without adversely affecting the epidermis (epidermal vaporization). But the efficacy of non-ablative laser treatments is less than ablative ones also. Fractional photothermolysis principle (FP) showed up after that in the history after with the idea of the generation of the multiple small thermal wounds at the skin directly (Manstein, Herron, Sink, Tanner, & Anderson, 2004). These small wounds were called microscopic treatment zones (MTZs). The FP technology was applied with both ablative and non-ablative laser treatments and widespread used today.

When the fractional radiofrequency microneedle (FRM) was launched. It commonly used for acne scar and large pore treatment. It was minimally invasive device (Cho, S. I. et al., 2012). This concept is different from the others RF device because the very fine microneedle is equipped at the applicator to deliver radiofrequency into accurate depths of the skin (Chaned, 2013).

In this research had study for others effect from FRM by treated the participants for 3 times in 4 weeks-interval full face and compared the result by the picture from VISIA, comparing side effects and volunteers' satisfaction.

Objective

To study about the clinical efficacy and other effect of the fractional radiofrequency microneedle device on acquired facial skin laxity in Thai people.

Materials and Methods

Twenty-five participants with facial laxity problem age between 25 – 55 years old were enrolled then treated with fractional insulated microneedle bipolar radiofrequency at week 0, 4th and 8th and patients were followed up at week 4th, 8th and 12th. Clinical outcomes were evaluated by compare a photo, which took by VISIA, between before and after each treatment by 3 independent doctor and also volunteer, skin elasticity was evaluated by Cutometer MPA580 between before and after each treatment and volunteer's satisfaction was evaluated by questionnaire after treatment. Adverse events and patient's satisfaction were recorded.

Statistic for Data Analysis

SPSS (IBM, Chicago, IL, USA) was used for the statistical analysis. Volunteers' research profile data used descriptive statistical analysis to provide descriptive information, such as percentages, means, modes, medians, ranges, standard deviations. Comparisons each parameter of subjects at baseline, 4, 8 and 12 weeks. Comparison of the Global aesthetic improvement scores by three masked physicians between before and after treatment each follow-up, R0 and R2 scores from cutometer. All data were tested using analysis with Paired T-test statistics or ANOVA repeated test. Volunteer's satisfaction at 12 weeks (4th week after completed treatment) follow-ups and others side effects use descriptive statistical analysis, the researcher did the following at significance levels of p-value <0.05

Results

Among the 25 enrolled participants, 21 subjects completed the study. The subjects were female 100%. The average subject age was 33.71± 5.98 years old. The clinical photographs were evaluated by three physicians blinded to treatment group and timing of the photographs, using Global Aesthetic Improvement Scale (GAIS) (1 = Worse, 2 = no change, 3 = improved, 4 = much improved, 5 = very much improved), and by participants themselves. The results from each physician with median score were compared between baseline and 4th, 8th and 12th week revealed significant by Pair T-test statistic (Figure 1) , on the other hand, The GAIS result by participants were increase in each visit but compared between baseline and 4th, 8th and 12th week revealed significant by Pair T-test statistic. (Figure 2)

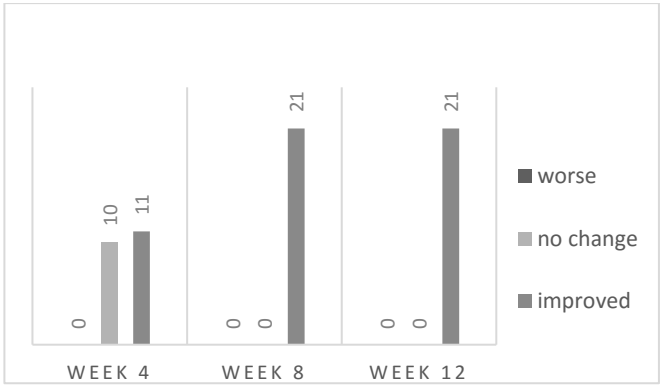


Figure 1. Bar graph compare the result of Median Global Aesthetic Improvement Scale between baseline and 4th, 8th and 12th week follow-up by 3 blinded physicians.

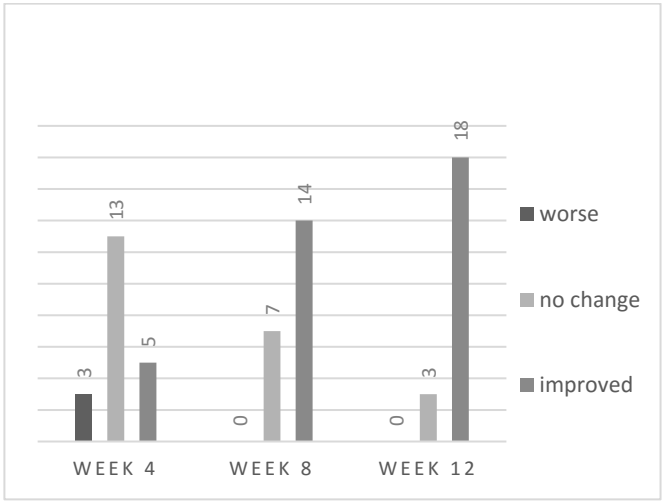


Figure 2. Bar graph compare the result of Median Global Aesthetic Improvement Scale between baseline and 4th, 8th and 12th week follow-up by participants.

There were statistical significance ($p<0.05$) had increase passive behavior of the skin to force (firmness) on FRM treatment at 8th week ($p<0.05$) and 12th week ($p<0.05$). There were statistical significance ($p<0.05$) had increase ability of returning (gross elasticity) at 12th week ($p<0.05$) (Figure 3).

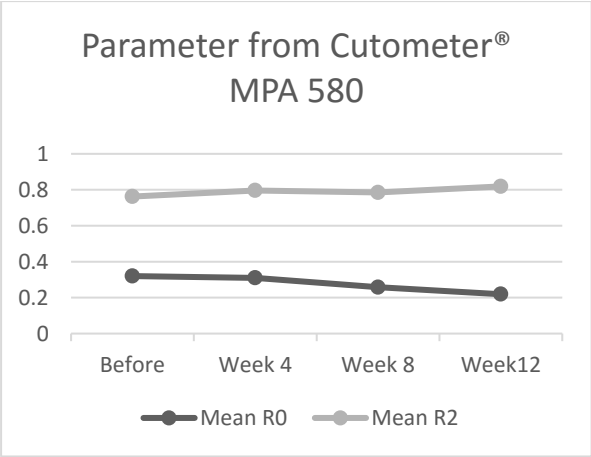


Figure 3. Linear graph show mean R0, R2 parameter from Cutometer®MPA 580

All volunteers indicated more pain and all bleeding in FRM treatment. Their common immediate side effects were erythema and swelling; post-inflammatory hyperpigmentation (PIH) and acne occurrence at nearby follow-ups. At four week after complete treatment, 12th week, volunteers had most scores for improvement of facial tightening as "very satisfied" 9%, "satisfied" 24%, "somewhat satisfied" 38%, "natural" 29%, that equals 71.43% of participants (15/21) satisfied facial tightening with using FRM device (Figure 5)

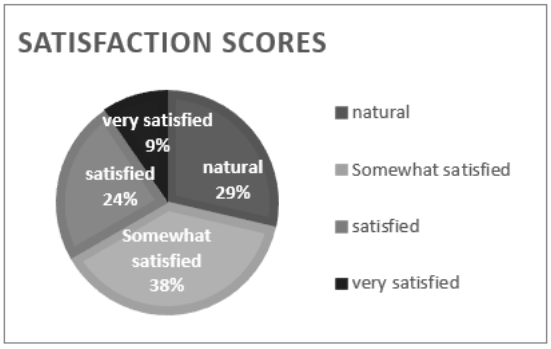


Figure 4. Descriptive satisfaction scores with using FRM device

Discussion

The results of this study confirmed that FRM facial skin tightening treatment was safe and effective treatment for full face.

According to the study, FRM gave clinical improvement since first time follow-up at 4th week. It is possible that in this study gave 3 passes within treatment, even though, in this study using coating needle, but when needle penetrated into skin FRM created thermal effect onto the superficial skin, so rejuvenating effect came faster. However using of microneedle be able to transfer heat into deeper dermis, it may help for collagen remodeling simultaneously.

Moreover, the improvement for skin laxity, this study showed that the face using FRM were firmer than baseline at 4th by mean parameter and significant improvement by statistic at 8th week, while skin elasticity had statistical improvement at 12th week. For such difference, it might be caused FRM can transfer energy to deeper dermis. The depth of energy transfer of FRM was more than 2000 μm due to microneedle length, result in abundant collagen production. When skin had more firmness it likely had not much skin elasticity.

Immediate side effects of this treatments were temporary. All volunteers indicated pain and all bleeding in every time follow-up, burning sensation also complaint. The common immediate side effects were erythema and swelling. Long-term side effects of both treatments such as hyperpigmentation and acne occurrence were similarly.

After 12 weeks, 4 weeks after complete treatment, participants had most satisfaction scores as "natural" 28.57% of participants and meaning of "satisfied", somewhat satisfied, satisfied and very satisfied, for 71.43%. We could infer that people thought variously for results and satisfaction did not always concomitant with the result.

Objective measurement such as melanin index, sebum excretion rates and trans-epidermal water loss should be measured in next studies.

Summary

FRM treatment can be effective treatments for skin tightening. Cutometer found more firmness of the skin at 4 week follow-up. The common side effects are erythema, swelling, post-inflammatory hyperpigmentation and acne occurrence. Pain feeling with and bleeding were indicated immediately after treatment. Longer follow-ups about long lasting result for next studies should be done and/or comparative studies with others device for better result of skin tightening effect in future.

References

Cho SI, Chung BY, Choi MG, Baek JH, Park CW, Lee CH, Kim HO. (2012). Evaluation of the clinical efficacy of fractional radiofrequency microneedle treatment in Acne scars and large pores. Dermatol Surg.2012 Jul;38 (7 Pt1):17-24

Kyu YS, Moon SY, Dong HK, Hee JL. (2012). Skin Rejuvenation by Microneedle Fractional Radiofrequency Treatment in Asian Skin; Clinical and Histological Analysis. Lasers in Surgery and medicine 44:631-636.

Nobuharu K, Kei N, Yukiko T, Kaori T, Shingo W. (2005). Non-ablative skin tightening with

radiofrequency in Asian skin. Lasers in Surgery and medicine 36:92-97

Prizker RN1, Robinson DM2. (2014) Updates in noninvasive and minimally invasive skin tightening. Semin Cutan med Surge.33, 182-187

Ryan MG, Jermy BG. (2014). Skin TighteningTechnologies. Facial Plast Surg ;30:62-67

Woolery-Lloyd H1, Kammer JN. (2011). Skin tightening. Curr Probl Dermatol.;42:147-152.

Yohei T. (2015). Long-Term Three-Dimention Volumetric assessment of Skin Tighing Using a sharply Tempered Non-Insulated Microneedle Radiofrequency Applicator With Novel Fractionated Pulse Mod n Asians. Lasers in Surgery and medicine.

The Efficacy and Safety of Topical Clitoria Ternatea Extracts for Eyebrow Enhancement in Thai People

Sasiprapa Petchnumnual[1], Tanomkit Pawcsuntorn[2]*

[1, 2] *School of Anti-Aging and Regenerative Medicine, Mae Fah Luang University, Bangkok, Thailand*
**Corresponding author. E-mail: tanomkit@yahoo.com*

Abstract

Eyebrow hypotrichosis is a common condition in Thailand and there is currently no standard treatment. Clitoria ternatea is one of the Thai traditional herbs that have been used for hundreds of years to increase thickness of eyebrow. However, there has been no previous report of efficacy and safety of topical Clitoria ternatea for eyebrow enhancement. **Objective:** The purpose of this study is to determine the efficacy and safety of topical 5% Clitoria ternatea serum for eyebrow enhancement in Thai people. **Materials and Methods:** Thirty participants (men and women, aged 20-60 years old) with idiopathic eyebrow hypotrichosis were enrolled and assigned 5% Clitoria ternatea serum on both eyebrows twice daily for 16 weeks. The subjects were followed every 4 weeks. Primary endpoint for efficacy evaluation was global photographic score and the secondary end-points were the changes in diameter and number of eyebrow hairs, subject satisfaction and side effects. **Results:** Among the 30 enrolled participants, all subjects completed the study. The subjects were female (19/30, 63.33%) and male (11/30, 36.67%). The average participant age was 31.97 ± 9.74 years old. The results revealed global photographic scores, eyebrow hair diameters and numbers were superior at follow-up compared with the baselines. However, only eyebrow hair diameter and number had statistically significance. Most of the participants (23/30, 76.67%) were satisfied with this therapy. The adverse events were temporarily mild pruritus on the treatment areas which can be resolved spontaneously in few days. **Conclusion:** 5% Clitoria ternatea serum can increase the eyebrow hair diameters and numbers with no serious side effect, however, the external appearances are not significantly changed. Further study should be conducted by compare the serum with placebo, increase concentration of the serum, or extend follow up period with more participants.

Keywords: Topical Clitoria ternatea/ Eyebrow/Enhancement/ Hypotrichosis/ Butterfly pea

Introduction

Eyebrow hypotrichosis does not only disrupt its function of protecting the eyes from dust and sweat but also has a great impact on a person's self-confidence, and quality of life. The thin eyebrow can be caused by various conditions such as skin diseases, endocrine abnormalities, autoimmune conditions, infection, tumor, trauma, genetic, or idiopathic.

Nowadays there is no standard treatment for idiopathic eyebrows hypotrichosis. Many doctors prescribe topical minoxidil, or topical bimatoprost to treat this condition and have good results. However, these drugs have several undesirable side effects: for example, local irritation, itching, dryness and erythema.

Clitoria ternatea commonly known as Butterfly pea belonging to the family Fabaceae and subfamily Papilionaceae. Many parts of this plant have been used for centuries as a memory enhancer, antidepressant, anticonvulsant, tranquilizing, and hair treatment including eyebrow enhancement. Previous study showed that Clitoria ternatea was the potent 5α-reductase inhibitor and hair growth promoter.

In addition, another study found that it can stimulate the melanogenesis. Nevertheless, there has been no previous report about efficacy and safety of topical Clitoria ternatea for eyebrow enhancement.

Materials and Methods

Objective

The purpose of this study is to determine the efficacy and safety of topical 5% Clitoria ternatea serum for eyebrow enhancement in Thai people.

Research methodology

Sample

Thirty participants (men and women, aged 20-60 years old) with idiopathic eyebrow hypotrichosis or willing to enhance their eyebrows were enrolled in this study. None of them used any systemic or topical drugs for eyebrow enhancement within six months.

Study design

The present study was approved by the Ethics Committee of Mae Fah Luang University. Patients were assigned treatment with 5% Clitoria ternatea serum on both eyebrows. Serum was applied twice daily for sixteen weeks. Follow-up visits were scheduled every four weeks

Efficacy assessment

The primary endpoint for efficacy evaluation was the score judged from global photographs by three doctors blinded to the treatment. And the secondary endpoints were the changes in diameter and number of eyebrow at the end of week 16th from baseline. In addition, subject satisfaction and side effects were also evaluated.

Global photographic assessment

Global photographs were taken using Visia (Canfield Scientific, Fairfield, NJ, USA). Three doctors who were blinded to the treatment conducted clinical assessments using 7-point scale, -3: greatly decreased, -2: moderately decreased, -1: slightly decreased, 0: no change, +1: slightly increased, +2: moderately increased and +3: greatly increased.

Hair diameter and number

A landmark for hair diameter and number measurement was the vertical lines drawn up from the mid-pupil. A single evaluator, who was blinded to the treatments, measured eyebrow hairs within 0.34 cm^2 areas using a Folliscope (LeadM, Seoul, South Korea). Eyebrow hair diameter was evaluated by measuring every eyebrow hair on the picture and then calculating the mean diameter. Eyebrow hair number was assessed by counting every hair on the picture.

Subject's satisfaction

The subject's satisfaction was evaluated by rated their perception of the result compared to baseline using a 7-point scale as global photographic score.

Safety evaluation

Safety evaluation was assessed from detailed history and physical examination. Subjects were asked whether they had itching, burning, redness, or scaling. The doctor examined eyebrows for erythema, scaling, edema or vesicles. Side-effects were rated as mild, moderate or severe.

Statistical Analysis

Changes in global photographic assessment and number of eyebrow hairs between baseline and post-treatment were compared using Friedman test. The repeated measure ANOVA was used to compare eyebrow hair diameter between pre- and post-treatment. In addition, the subjects' satisfaction and the number of subjects who developed adverse events were described by using descriptive analysis. All the results were considered statistically significant at $P < 0.05$.

Results and Discussion

Baseline characteristics

Among the 30 enrolled subjects, all completed the study. The majority of subjects were female (19/30, 63.33%). The average age was 31.97 ± 9.74 (range, 20-59) years.

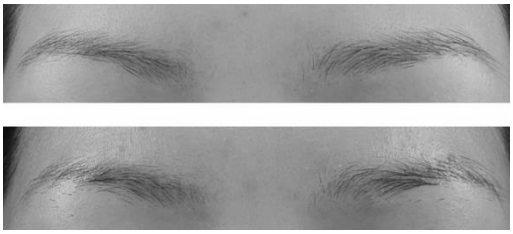
Global photographic assessment

Median of all global photographic scores were collected in table 1. At week 4th, 14 of 30 (46.67%) subjects had slightly increased eyebrow hairs (score +1), and 14 of 30 (46.67%) had no changed (score 0). After 16 weeks, the number of subjects who had slightly increased eyebrow hairs (score +1) were raised to 16 of 30 (53.3%), and who had no changed (score 0) were decreased to 11 of 30 (36.7%). However, there were no statistically different among these changes. (p-value = 0.067)

Table 1: Median of all global photographic scores

Week	Global photographic score				Total
	-1	0	+1	+2	
4	2	14	14	0	30
8	2	11	16	1	30
12	1	12	15	2	30
16	1	11	16	2	30

Figure 1: This picture demonstrates improvement of eyebrow growth before (a) and 16 weeks after (b) treatments



Eyebrow hair count

The changes in eyebrow hair numbers between each follow-up week were statistically difference ($P < 0.001$) from Friedman test. Thus, we conducted the post-hoc test using Wilcoxon signed-rank tests and the result was shown in Table 2. Number of eyebrow hairs were significantly increased from baseline at week 12th and 16th. ($P < 0.05$).

Table 2: Mean changes of eyebrow count from baseline

Week	Mean of eyebrow count	p-value
0	31.23	-
4	31.03	0.619
8	31.73	0.310
12	32.97	0.015*
16	34.20	0.000*

*Wilcoxon Matched-Pairs Signed Ranks test, $P<0.05$

Eyebrow hair diameter

Mean changes of eyebrow diameter from baseline are shown in Table 3. The eyebrow diameter was significantly increased from baseline after 8 weeks of treatment ($P = 0.021$). At week 16th, mean difference from baseline was 4.367 μm. ($P < 0.001$)

Table 3: Mean changes of eyebrow diameter from baseline

Week	Mean of eyebrow diameter	Mean Difference (Week 0 – Week i)	p-value
0	65.80	0	-
4	66.60	-0.800	0.713
8	67.47	-1.667*	0.021*
12	68.40	-2.600*	0.002*
16	70.17	-4.367*	0.000*

* Repeated measure ANOVA, P<0.05

Subject satisfaction

Most of the subjects were slightly satisfied (+1) with the result (12/30, 40%). There is only one subject who slightly unsatisfied (-1) with this treatment (3.3%).

Safety evaluation

The incidence of adverse events was 10% (3/30). All of them reported mild itching. However, physical examination showed no rash, or other abnormal skin lesions.

Discussion

The Clitoria ternatea is the potent 5α- reductase inhibitor and can promote the hair growth. But the effect of Clitoria ternatea on the eyebrow is still unclear.

From this research, we found that topical Clitoria ternatea can increase the eyebrow hair diameters and numbers. This effect of Clitoria ternatea is in accord with previous study by Kumar N.et al. (Kumar N.et al., 2012). They also found that Clitoria ternatea can inhibit 5α-reductase enzyme and stimulates hair growth.

At baseline, the mean hair count was about 31.2 hairs/0.34 cm^2. Until week 12^{th}, it was significantly increased to 32.9 ($p = 0.015$). For the aspect of hair diameter, after 4, 8, 12 and 16 weeks, the mean changes in hair diameter were 0.8, 1.7, 2.6 and 4.3 μm, respectively. The statistical significance was appeared after 8 weeks. However, the global photographic score was not significantly increased. It is possible that the evaluation by folliscope is more sensitive than photograph.

Although the appearance was minimally different, most of the subjects (76.67%) were satisfied with the result. It implies that a little change can make a big difference for the patients with eyebrow hypotrichosis. Besides, the Clitoria ternatea serum was safe. Only 3 of 30 subjects (10%) complaint of mild itching without any skin lesion and there was no other serious complication.

This study still has some limitation. Therefore, we suggest that further studies should be conducted to valuate the higher concentration of the serum, compare with placebo, longer follow- up period with more participants. In addition, the effect of Clitoria ternatea on the eyebrow also should be investigated.

Conclusions

Eyebrow can protect the eye from the sweat and dust and give the confidence for the human. Clitoria ternatea is a hair promoter, however, the effect on eyebrow is unclear. Although 5% Clitoria ternatea can enhance the eyebrow hair when measured by the folliscope, it was not enough to distinguish from the photograph. The diameter and hair count of the eyebrow were significantly increased, but the global photographic score was slightly increased without the significance. It was possible from the inadequate concentration or dosage. Further study should be investigated.

References

MD, J. V. (2014, January). The Biology, Structure, and Function of Eyebrow Hair. Journal of drugs in dermatology, 13(1).

Carruthers, J., & Carruthers, A. (2014, January). Social Significance of the Eyebrows and Periorbital Complex. Journal of drugs in dermatology, 13(1).

Kumar, N., Rungseevijitprapa, W., Narkkhong, N.-A., Suttajit, M., & Chaiyasut, C. (2012). 5 alpha-reductase inhibition and hair growth promotion of some Thai plants. Journal of Ethnopharmacology, 765– 771.

Mukherjee, P., Kumar, V., Kumar, N., & Heinrich, M. (2008). The Ayurvedic medicine Clitoria ternatea - from traditional use to scientific assessment. J Ethnopharmacol, 291-301.

กฤตติญารัตน์ สมวงศ์และชุตินันท์ ประสิทธิ์ภูริปรีชา. (2012). ฤทธิ์ต้านออกซิเดชัน และฤทธิ์กระตุ้นการสังเคราะห์เม็ดสีเมลานินของสารสกัดสมุนไพรไทยพื้นบ้านบางชนิด เพื่อใช้สำหรับผมหงอกก่อนวัย. The 4th Annual Northeast Pharmacy Research Conference of 2012 “Pharmacy Profession in Harmony”, (หน้า 125-134).