A CLINICAL, PROSPECTIVE, RANDOMIZED, DOUBLE-BLIND TRIAL COMPARING 1% ALPHA ARBUTIN WITH 3% HYDROQUINONE IN THE TREATMENT OF MELASMA.

A THESIS

BY

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Presented in partial fulfillment of the requirements

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has approved by The Graduate School as partial fulfillment of the requirements for the Master of Arts degree in Dermatology of Srinakharinwirot University.

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Teerasak Moryadee

The author

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Melanocytes, the specialized melanin-producing cells located at the basal layers of the epidermis [2]. They form long slender processes that ramify among neighboring cells called keratinocytes. In this way, each melanocyte makes contact with around 30-40 keratinocytes and this constitutes the epidermal-melanin unit (Figure 2). The melanocyte has receptors on its surface that specifically interlock with a natural hormone called melanocyte stimulating hormone or MSH. These receptors, when were activated by MSH , they trigger the cell to make the dark pigment called melanin.

Melanin is a generic term that refers to a group of complex biopolymers [3]. The chemical composition and physical properties are highly dependent on how and where it was formed.

There are two classes of melanins present in human skin, eumelanins and phaeomelanins. Eumelanin is the dark brown to black pigment which is the form of melanin protective against UV radiation. Phaeomelanin is a red-yellow pigment which is the form of melanin associated most closely with the potential to sunburn and maybe to develop skin cancers. Individuals with light coloured skin and brown, blond or red hair tend to have a significant amount of phaeomelanin whereas, darker skinned and black haired individuals have predominantly eumelanin.

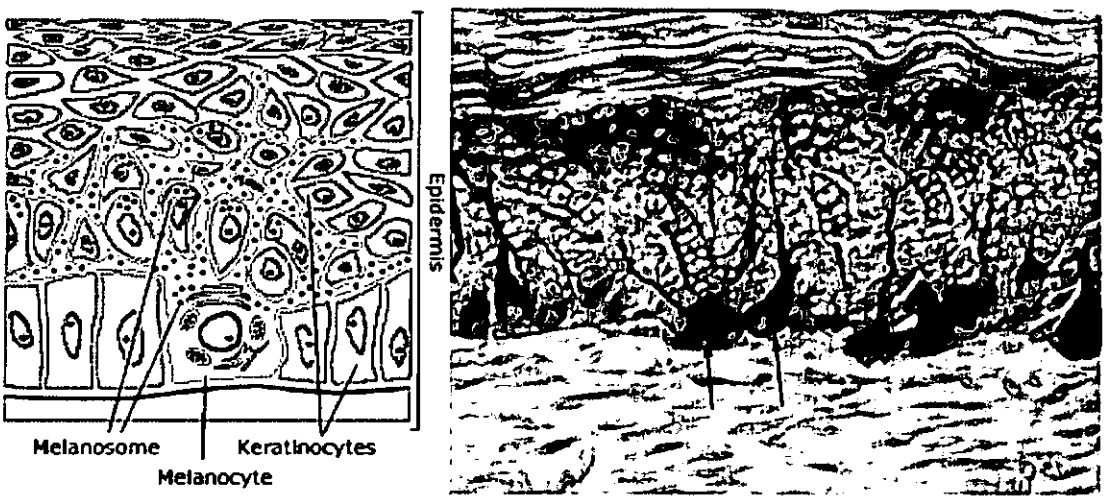


Figure 2 Epidermal-melanin unit.

Chapter 1

Introduction

1.1 Melasma

Melasma is a commonly acquired increasing of pigmentation that occurs exclusively in sun-exposed areas which appear as brownish color. It is exacerbated by sun exposure, pregnancy, oral contraceptives and certain anti-epilepsy drugs. Melasma is common, especially in women of child-bearing age (90%) [1]. However, up to 10% of cases have been reported in males. While all races are affected, there is a prominence in Latinos and Asians. Melasma is more apparent during and after periods of sun exposure and less obvious in winter months, when sun exposure is lacking.

Melasma presents in one of the three symmetrical facial patterns. The most common is a centrofacial pattern, involving the cheeks, forehead, upper lip, nose and chin (Figure1). Less common are the malar pattern, involving the cheeks and nose, and the mandibular pattern, involving the ramus of the mandible.

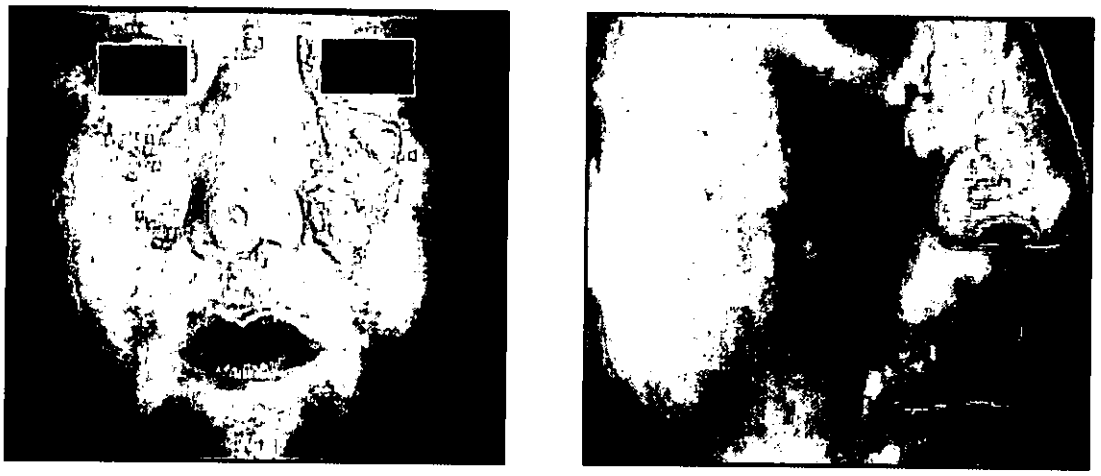


Figure1 Clinical picture of melasma.

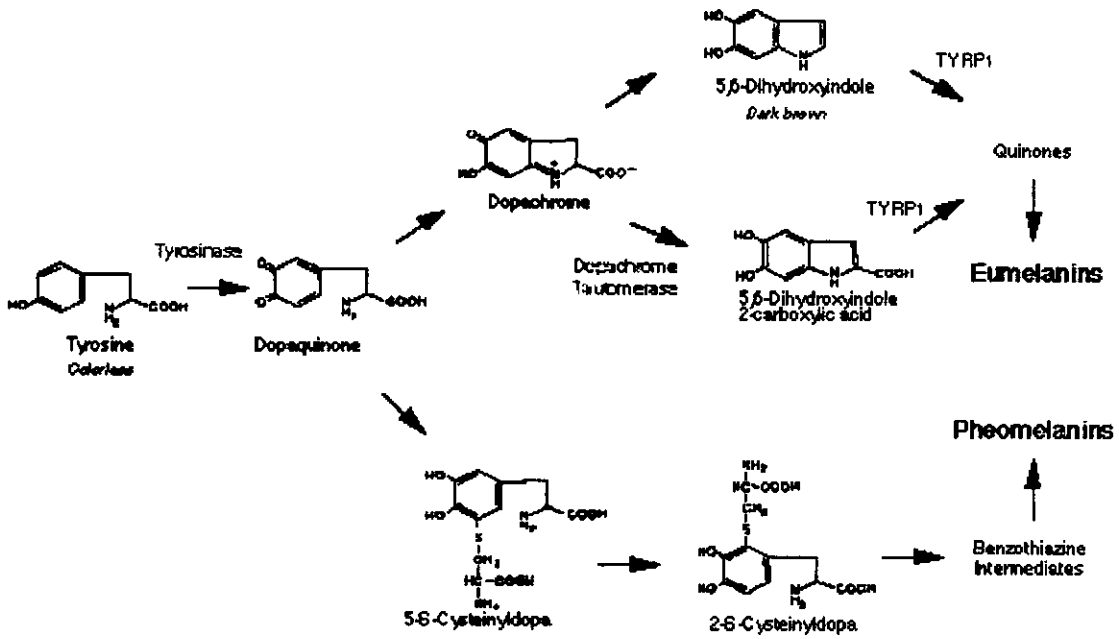


Figure 3 Melanogenesis pathway

1.3 Classification of melasma.

Melasma can be clinically classified into 3 types as follows: epidermal type, dermal type and mixed type.

1) **Epidermal type:** light brown color : the histopathology shows melanin is in the basal and suprabasal layer of the epidermis.

2) **Dermal type** : grayish color : the histopathology shows melanophages loaded with melanin in the papillary dermis.

3) **Mixed type:** dark brown color : the histopathology shows melanin is in the basal layer of epidermis and in papillary dermis

In all types, there are the moderate increase in the number of melanocytes and also in the cellular activity. [4]

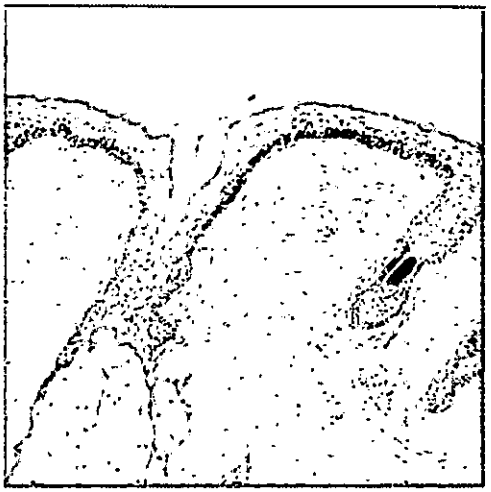


Figure 4 Histopathology of melasma (epidermal type)

1.4 The causes of melasma

The causes of melasma may derive from multifactor [5], including pregnancy, oral contraceptives, endocrine dysfunction, genetic factors, medications, nutritional deficiency, hepatic dysfunction, and other factors. The majority of cases appear to be related to pregancy or oral contraceptives. The infrequency of melasma in postmenopausal women on estrogen replacement suggests that estrogen alone is not the cause. In more recent experience, combination treatment using estrogen plus progestational agents is being used in postmenopausal women, and melasma is being observed in some of these older women who did not have melasma during their pregnancies. Sun exposure would appear to be a stimulating factor in predisposed individuals. Although a few cases within families have been described, melasma should not be considered a heriditary disorder.

1.5 The management of melasma

The management of melasma is a challenge, as there is no gold standard treatment and recurrences are common. The principles of therapy are based on the following strategies 1) sunscreen use 2) inhibition the activity of melanocytes 3) removal of melanin 4) dispersion of melanin granules

Melasma is difficult to treat. This is confirmed by the enormous variety of options for treatment, which range from conventional treatment including elimination of any possible causative factors and coupling with use of a sunscreen and hypopigmenting agents and often in combination with other therapies such as tretinoin, topical corticosteroids, superficial peeling agents and laser treatment. (6-9)Despite these measures, treatment of this recalcitrant disorder is often difficult and frustrating for the patient and the doctor.

1.6 Aim of the thesis

Due to the paucity of the study about the efficacy and safety of alpha arbutin in the treatment of melasma in female Asian population. Then we conducted this double-blind, randomized, prospective, clinical study aim to evaluate the efficacy and side effect of the 1% alpha arbutin versus a standard depigmenting agent,3% hydroquinone in the treatment of facial melasma in Thai women.

Chapter 2

Review literature

Many modalities of treatment for melasma are available including chemical agents or physical therapies, but none are completely satisfactory. Depigmenting compounds should act selectively on hyperactivated melanocytes, without short- or long-term side-effects, and induce a permanent removal of undesired pigment. Since 1961 hydroquinone, a tyrosinase inhibitor, has been introduced and its therapeutic efficacy demonstrated, and other whitening agents specifically acting on tyrosinase by different mechanisms have been proposed. Compounds with depigmenting activity are now numerous and the classification of molecules, based on their mechanism of action, has become difficult. Systematic studies to assess both the efficacy and the safety of such molecules are necessary. Moreover, the evidence that bleaching compounds are fairly ineffective on dermal accumulation of melanin has prompted investigations on the effectiveness of physical therapies, such as lasers

2.1 Whitening agents (depigmenting agents)

The ideal whitening agents should have a potent, rapid and selective bleaching effect on hyperactivated melanocytes, carry no short- or long-term side-effects and lead to a permanent removal of undesired pigment, acting at one or more steps of the pigmentation process.

Depigmentation can be achieved by regulating 1) the transcription and activity of tyrosinase, tyrosinase related protein-1 (TRP-1), tyrosinase related protein-2 (TRP-2), and/or peroxidase; 2) the uptake and distribution of melanosomes in recipient keratinocytes and 3) melanin and melanosome degradation and turnover of 'pigmented' keratinocytes .As a result of the key role played by tyrosinase in melanin biosynthesis, most whitening agents act specifically to reduce the function of this enzyme by means of several mechanisms: 1) interference with its transcription and/or glycosylation, 2) inhibition by different modalities, 3) reduction of by-products and 4) post-transcriptional control.

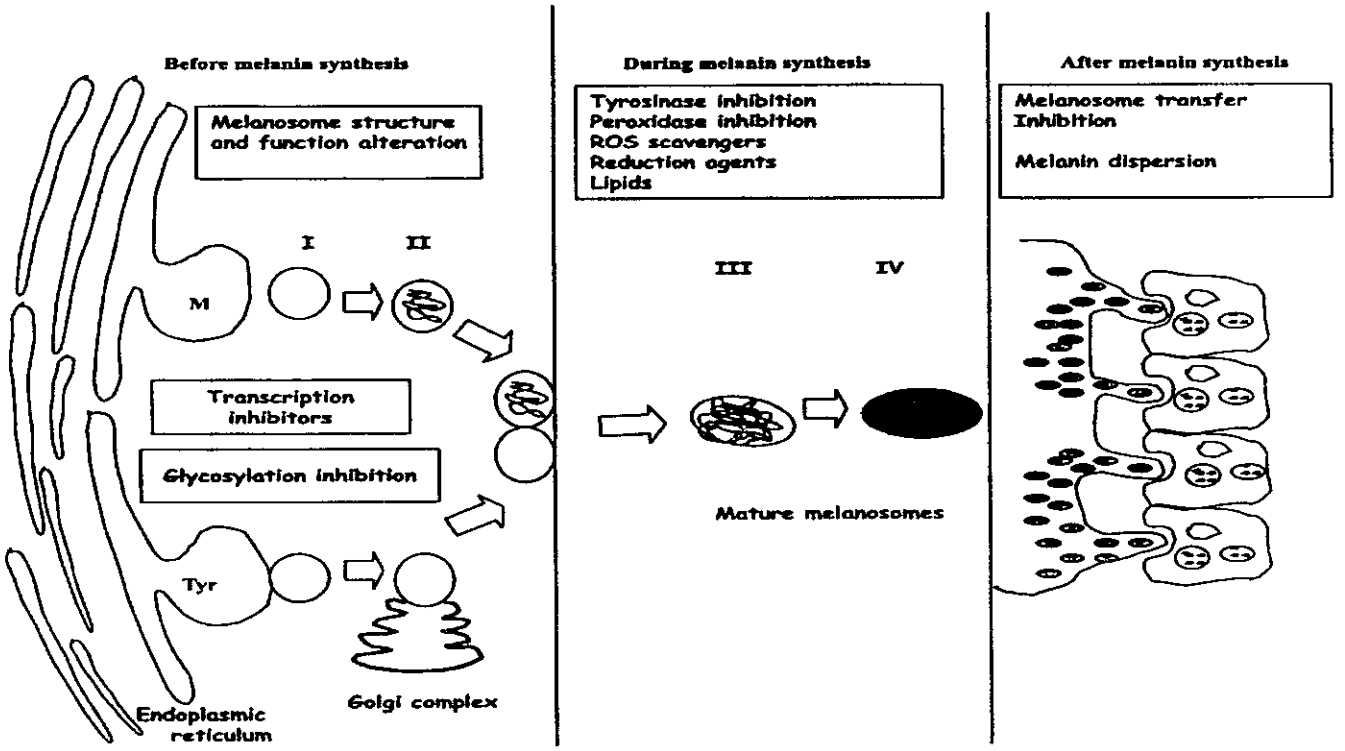


Figure 5 Schematic illustration of the possible approaches to interfere with melanogenesis pathway. Tyr, tyrosinase ; M, melanosomes; ROS, reactive oxygen species.

2.2 Regulation of melanogenic enzymes

2.2.1 Control of Tyrosinase Activity

The classification of tyrosinase inhibitors is difficult because of the capability of several compounds to work in different ways, interacting with both catalytic and regulatory sites of the enzyme or being metabolized to a product that can act as either a non-competitive or a competitive inhibitor. Tyrosine hydroxylation is the rate limiting step for melanogenesis, but requires l-3,4-dihydroxyphenylalanine (l-DOPA), the substrate of tyrosinase's second activity, as a cofactor. Thus, tyrosinase activity cannot be easily examined through kinetic analyses. Moreover controversial results were obtained when purified mushroom tyrosinase or cell lysates of melanoma cells were employed. Most of the inhibitors are phenol/catechol derivatives, structurally similar to tyrosine or DOPA, which act as alternative substrates of tyrosinase without producing pigment [10,11]

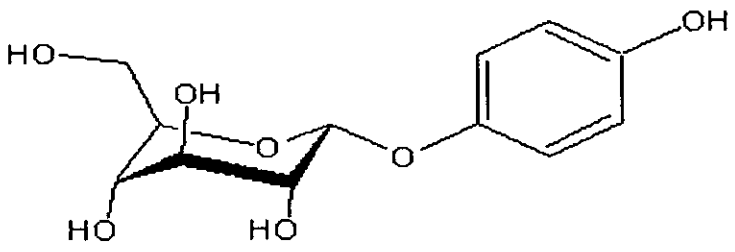
Phenolic Substrates

Hydroquinone

The most popular depigmenting agent is hydroquinone (dihydroxybenzene; HQ) introduced for clinical use since 1961. Several trials have demonstrated its therapeutic efficacy alone or in association with other compounds [12] HQ seems to exert its effects mainly in melanocytes with active tyrosinase activity, such as epidermal hyperactivated melanocytes [13] and has been considered as a reference standard in evaluating depigmenting agents [14,15]. The oxidation products are quinones and reactive oxygen species (ROS), which lead to an oxidative damage of membrane lipids and proteins, including tyrosinase, and depletion of glutathione contributes to the lightening action . HQ may interfere with pigmentation even through: (i) the covalent binding to histidine or interaction with coppers at the active site of tyrosinase (ii) the inhibition of DNA and RNA synthesis and (iii) the alteration of melanosome formation and melanization extent . However, because of the hazard of long-term treatments , the use of hydroquinone in cosmetics has been banned by the European Committee (24th Dir. 2000/6/EC) and formulations are available only by prescription of physicians and dermatologists

Adverse reactions from hydroquinone use include irritant and allergic contact dermatitis, ochronosis and nail discoloration. Postinflammatory hyperpigmentation may occur from the contact dermatitis. Hypopigmentation of the normal skin surrounding the treated areas may also occur. These usually resolve with the discontinuation of the hydroquinone treatment.

Arbutin,



Arbutin

Figure 6 Structure of Arbutin

Arbutin, which is the D-glucopyranoside derivative of hydroquinone, is a naturally occurring plant derived compound that has been used for postinflammatory hyperpigmentation.(16). Arbutin is found in the bark and leaves of various plants, usually occurring together with methylarbutin. Naturally occurring Arbutin was first characterized by Kawalier who obtained it from bearberry leaves. It is also found in the leaves of blueberry, cranberry, and pear. Synthetic Arbutin was first reported by Mannich3and later by others [4].

Arbutin is effective in the treatment of disorders of hyperpigmentation characterized by hyperactive melanocytes[16] The action of arbutin is dependent on its concentration. Higher concentrations are more efficacious than lower concentrations, but they may also result in a paradoxical hyperpigmentation. In comparative in vitro studies of various compounds used to improve the appearance of disorders of hyperpigmentation, arbutin was found to be less toxic than hydroquinone. A dose-dependent reduction in tyrosinase activity, as well as melanin content in melanocytes, was also demonstrated [17] The study mechanism of its action in human melanocyte culture, Arbutin inhibited the tyrosinase activity of cultured human melanocytes at noncytotoxic concentrations. It did not affect the expression of tyrosinase mRNA. Melanin production was inhibited significantly by arbutin.These results suggest that the depigmenting mechanism of arbutin in humans involves inhibition of melanosomal tyrosinase activity, rather than suppression of the expression and synthesis of tyrosinase.

Study of inhibitory effect of arbutin (hydroquinone-beta-D-glucopyranoside) on the melanogenesis was studied biochemically using cultured B16 melanoma cells. The maximum arbutin concentration lacking an inhibitory effect on cell growth was 5 X 10(-5) M. At this concentration, melanin content per cell was decreased significantly to about 39%, compared with that of arbutin untreated cells. Also, tyrosinase activity of arbutin treated cells was decreased significantly. When arbutin was added to B16 melanoma cell suspension, arbutin was not hydrolyzed to liberate hydroquinone. Further, tyrosinase activity in crude preparations from B16 melanoma cells was inhibited by arbutin. From these results, it is suggested that arbutin can inhibit the melanogenesis by affecting not only the synthesis but also the activity of tyrosinase rather than by killing melanocytes B16 melanoma cells [18]

Alpha arbutin

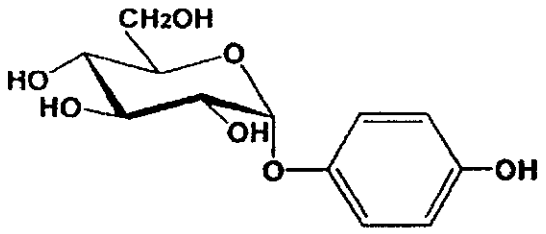


Figure 7 Structure of Alpha-arbutin

Alpha arbutin(4-hydroxyphenyl alpha-glucopyranoside),the new derivative of hydroquinone. The studied of inhibitory effects of 4-hydroxyphenyl alpha-glucopyranoside (alpha-arbutin) on melanogenesis in cultured human melanoma cells, HMV-II, and in a three-dimensional cultured human skin model. alpha-Arbutin showed no inhibitory effect on HMV-II cell growth at a concentration below 1.0 mM. Melanin synthesis in cells treated with alpha-arbutin at 0.5 mM decreased to 76% of that in non-treated cells. The cellular tyrosinase activity of HMV-II cells also significantly decreased, while the expression of its mRNA was not affected. Melanin synthesis in a human skin model was also evaluated by the macro- and microscopic observation of its pigmentation as well as by quantitative

measurements of melanin. Treatment of the human skin model with 250 microg of alpha-arbutin did not inhibit cell viability, while melanin synthesis was reduced to 40% of that in the control. These results indicate that alpha-arbutin is an effective and safe ingredient for skin-lightening. [19]

Comparison of inhibitory effects of alpha-arbutin and beta -arbutin on human tyrosinase. The effects of 4-hydroxyphenyl alpha-glucopyranoside (alpha-arbutin) and 4-hydroxyphenyl beta-glucopyranoside (arbutin) on the activity of tyrosinase from human malignant melanoma cells were examined. The inhibitory effect of alpha-arbutin on human tyrosinase was stronger than that of arbutin. The K(i) value for alpha-arbutin was calculated to be 1/20 that for arbutin. The arbutin-alpha-glycosides exhibited competitive type inhibition on human tyrosinase. The arbutin-alpha-glycosides possessed stronger inhibitory activity than arbutin, but less activity than alpha-arbutin. These results suggested that the alpha-glucosidic linkage of hydroquinone-glycosides plays an important role in the inhibitory effect on human tyrosinase. [20]

Effects of alpha and beta-arbutin on activity of tyrosinases from mushroom and mouse melanoma. The effects of alpha- and beta-arbutin on the activity of tyrosinases from mushroom and mouse melanoma were examined. alpha-arbutin was synthesized from hydroquinone and starch using glucoside synthetase (GSase). beta-arbutin inhibited both tyrosinase activities from mushroom and mouse melanoma. alpha-arbutin inhibited only the tyrosinase from mouse melanoma, 10 times as strongly as beta-arbutin. The IC50 of alpha-arbutin was 0.48 mM and its inhibitory mechanism was speculated to be mixed type inhibition, while that of beta-arbutin was noncompetitive. [21]

A skin- lightening study by Pentapharm on 80 Chinese women demonstrated that an emulsion containing 1 % Alpha arbutin resulted in a faster and more pronounced skin lightening effect after 1 month when compared with other commonly used single components at 1% use levels. (Kojic acid , beta arbutin and hydroquinone). In another study by Pentapharm, a 3 months study on 26 females volunteers showed that 2 % Alpha arbutin in a cream formulation minimizes liver spots more efficiency than 2% Beta –arbutin.

Kojic acid

Kojic acid (5-hydroxy-2-hydroxymethyl-4H-pyran-4-one) is an antibiotic produced by many species of *Asperigillus* and *Penicillum*. The depigmenting action is attributed to the chelating ability[22] , even if an interference with different steps of melanin synthesis and inhibition of nuclear factor-kappa B activation in keratinocytes, contrasting with the hyperpigmentation associated with inflammatory response, have been described Clinical trials have reported a skin lightening effect of kojic acid [23]

Azelaic Acid

Compounds able to bind either amino or carboxyl groups may block the access of tyrosine to the active site, behaving as competitive inhibitors. Among these, azelaic acid (AZA), a naturally occurring 9-carbon dicarboxylic acid compound isolated from cultures of *Pityrosporum Ovale*, is included. AZA inhibits tyrosinase activity in vitro and may also interfere with DNA synthesis and mitochondria activity in hyperactive and abnormal melanocytes [24]. AZA has been used to treat melasma and postinflammatory hyperpigmentation and to arrest the progression of lentigo maligna to melanoma . Topical azelaic acid (15-20%) has been reported to be efficacious in improving melasma [25] even if not all the authors are in agreement with these therapeutic effects.

2.2.2 Redox Agents and ROS Scavengers

Ascorbic acid

Ascorbic (AsA) interferes with the different steps of melanization, by interacting with copper ions at the tyrosinase active site [26] and reducing dopaquinone and by blocking DHICA oxidation [27].However, AsA is highly instable, being quickly oxidized and decomposed in aqueous solution and, because of its prevalent hydrophilic nature, has a low degree of penetration into the skin.

2.2.3 Melanin Dispersion and Acceleration of Skin Turnover

The capacity of several compounds to disperse melanin pigment and/or accelerate epidermal turnover can result in skin lightening. Chemical substances used as exfoliantes, such as-hydroxy acids, free fatty acids and retinoic acid, stimulates cell renewal facilitating the removal of melanized keratinocyte, leading to melanin pigment loss [28]. Topical application has been shown to reduce the visibility of age spots, without reducing their size or number [29], and can be useful in the treatment of melasma [30]. Liquiritin, a flavonoid glycoside of liquorice, has been found to induce a significant improvement of hyperpigmentation in patients with bilateral and symmetrical idiopathic epidermal melasma [31]. The mechanisms proposed involve melanin dispersion by means of the pyran ring of its flavonoidal nucleus, and acceleration of epidermal renewal.

2.2.4 Transcriptional Control of Tyrosinase Expression

Transcription of genes encoding tyrosinase and TRP-1 is under transcriptional control of the microphthalmia transcription factor (MITF) Substances able to inhibit MITF expression and activity, as well as the extracellular signal-regulated kinase (ERK) and serine-threonine kinase (AKT)/protein kinase B (PKB) pathways, could represent depigmenting agents.

Among these," tretinoin" (all-trans retinoic acid; ATRA), acting on retinoid-activated transcription factors, interferes with melanocyte development and melanogenesis. ATRA has bimodal effects on melanocytes: it stimulates the differentiation of melanocyte precursors, inducing transcription of tyrosinase by protein kinase C (PKC) activation and MITF expression, and removes differentiated melanocytes by promoting apoptosis via caspase-3 pathway and bcl-2 down-modulation [32]. ATRA does not inhibit melanogenesis in skin equivalents or monolayer cultures of murine and human melanocytes whereas it enhances the pigmentation of low melanized (S91 murine) melanoma cells, and decreases that of highly pigmented normal human melanocytes after UV exposure [33]. Topical ATRA stimulates tyrosinase activity only in individuals with low skin type and improves the irregular hyperpigmentation associated with photo-aging or inflammation.

2.2.5 Inhibitors of Inflammation-Induced Melanogenic Response

Some mediators produced by keratinocytes after exposure to pro-inflammatory stimuli or UV exposure, such as interleukin-1α (IL-1 α) and endothelin 1 (ET-1), are able to promote melanogenesis. Therefore, anti-inflammatory compounds could be useful for the prevention or treatment of postinflammation hyperpigmentation. ET-1 shows an unique behavior in exerting stimulatory effects both on DNA synthesis and melanization by human melanocytes (34). Activation of epidermal ETs is determined by the enzymatic cleavage of inactive prepolypeptides by an endopeptidase termed ET converting enzyme (ECE), regulated by the pro-inflammatory cytokine IL-1 α (35). Topical application of M. chamomilla extract inhibits UVB-induced pigmentation, by avoiding ET-1-induced DNA synthesis, but not the IL- α-induced ET-1 production and tyrosinase activation.

Among anti-inflammatory drugs, topical corticosteroids have been included in several clinical trials for the treatment of melasma even to decrease the irritation caused by depigmenting agents [36-38] The possible mechanism is the suppression of cytokines through the inhibition of NF-kB activation, which may explain their short-lived effect with a transient loss of color. Glabridin, the main component of hydrophobic fraction of liquorice extracts decreases tyrosinase activity in B16 melanoma cells, without affecting DNA synthesis, and, in guinea-pigs, inhibits UVB-induced skin pigmentation, as well as erythema [39]. The capability to inhibit cyclooxygenase activity and superoxide anion production suggests that the anti-inflammatory effect involves an interference with the arachidonic acid cascade and that protection against oxidative stress has a key role in modulating melanogenesis. Variations in the chemical structure modulate the effect, because melanogenesis inhibition is lost by replacing both hydroxyl groups and reduced by substitution at the 4' position.

2.3 Laser Treatment of Pigmented Lesions

Therapeutic options, other than chemical agents, have been proposed for hyperpigmentation, such as cryotherapy with liquid nitrogen, laser surgery, chemical peeling and superficial dermoabrasion [40,41]. Since the first demonstration , treatments of pigmented lesions with argon laser, CO_2 laser, Nd:YAG laser or Q-switched ruby laser have been reported [42]. The theory of a selective photothermolysis suggests that laser therapy would allow discriminating destruction of pigment without injuring the surrounding tissue . Selective melanin photothermolysis can be obtained with any laser light having a wavelength in the absorption spectrum of melanin and sufficient energy levels to target melanosomes [43]. Laser induces extreme heating of melanosomes with subsequent thermal expansion, local vaporization and generation of acoustic waves that damage the nucleus and eventually destroy the pigment-laden cells. The released melanin is then removed through *trans*-epidermal elimination or phagocytosis by dermal macrophages. To be effective and specific, wavelengths that avoid absorption by other skin chromophores and penetrate to the desired depth have to be used. Different laser wavelengths induce similar melanosome alterations, but the threshold doses and penetration are substantially different. Shorter wavelengths (<600 nm) damage pigmented cells with lower energy fluences, while longer wavelengths (>600 nm) penetrate deeper into the skin but need more energy to induce melanosome disruption. Thus, short wavelengths damage only superficial pigmented lesions, leaving deeper structures intact, while longer wavelengths can target pigmented lesions in the dermis. Equally important is the pulse duration, which should be shorter than the thermal relaxation time of melanin. Clinically, the immediate effect of sub-microsecond pulses in pigmented skin is tissue-whitening, which corresponds to melanosome disruption and is an end-point during treatment. There are four main short-pulsed pigment-selective lasers in clinical use nowadays: the pigmented lesion dye laser (PLDL) (510 nm, 300 ns), the Q-switched ruby laser (694 nm, 24-40 ns), the Q-switched alexandrite laser (755 nm, 50-100 ns), and the Q-switched Nd:YAG laser (1064 nm, 5-10 ns), which can be frequency-doubled to emit a green light at 532 nm of the same pulse duration.

Epidermal melasma responds to laser treatment similar to the use of depigmenting agents or chemical peels, but the dermal and mixed types are resistant to laser therapy. However, several months after treatment with a Q-switched ruby laser, both epidermal repigmentation and an increased number of dermal macrophages have been observed [44]. The poor response may be referred to the hyperactivation of melanocytes and to the laser-mediated inflammatory response, that leads to rapid reoccurrence or even darkening of the treated areas. To improve the efficacy, the development of a combination laser treatment has been proposed. A pilot study has described the effectiveness of a combination laser therapy (pulsed CO_2 laser followed by Q-switched alexandrite laser) in treatment of dermal-type melasma [45]. Given the epidermal trauma and worsening in pigmentation induced by laser impact, it is not surprising that laser treatment of postinflammatory hyperpigmentation has been discouraging. [46].

Chapter 3

Materials and Methods

3.1 Patients

Forty two patients who came to visit Srinakharinwirot skin center with clinical complaint of melasma were selected by the following criteria.

Inclusion criterias

1. All patients wereThai women, age 20-60 years with facial melasma, epidermal and mixed type.
2. They had skin photo type III, IV, V. (Fitzpatrick 's classifications)
3. They could be follow up at week 0, 2, 4, 6, 8, 10.
4. They gave signed and informed consent prior to participation in the study.

Exclusion criterias

1. The ones that had active dermatologic disease.
2. The known cases of skin sensitivity to clarifying agents in study product.
3. The ones that had previous treatment of melasma over a period at least 1 month.
4. The ones who use of steroid , retinoic acid and contraceptive pill
5. The ones with pregnant , breast feeding women or who had history of endocrinopathies.

3.2 Ethical considerations

The study was evaluated and approved by the Committee of Ethics and Medical Research of the medical faculty Srinakarintaravirot University.

3.3 Methodology

Forty two patients received two tubes of cream, a standardized sunscreen [NO-AD sun block lotion sun protection factor (SPF) 30 ,Solar Cosmetic Labs. INC . Miami, Florida 33014 USA] and a facial cleansing foam for daily use.[Smooth E babyface foam Non-ionic ,Nature 's VITA].

All of the tubes had the same appearance . The cream had the same characteristics and identified as "right side" or" left side." The only person who knew what was being used by each patient on each side of the face was the pharmacist.
The selected patients were divided into 3 groups :

Group 1,(14 patients)the patients received one tube containing 3% hydroquinone cream and one tube containing placebo to be applied to opposite sides of the face at night.

Group 2 ,(14 patients) the patients received one tube containing 1% alpha arbutin cream and one tube containing placebo to be applied to opposite sides of the face at night.

Group3, (14 patients) the patients received one tube containing 1% alpha arbutin cream and one tube containing 3 % hydroquinone cream , to be applied to opposite sides of the face at night.

3.3.1. Baseline evaluation

Baseline evaluation includes history of melasma, length of time present , relate to pregnancy, hormonal therapy, sun exposure, cosmetic use, previous treatment , family history of melasma.

Clinical type of melasma was identified for epidermal or dermal type by Wood's lamp .

3.3.2. Photographic documentation

Professional photographer took photographs before and after treatment, (week 0, 10). Three angles were standardized for photographing the face (front, left and right) for each patient. (Figure 8)

At the end of the study ,the photo of patients at baseline and at 10 weeks were assessed by two doctors with a three-point scale as follows : good improvement (more than 61%), fair (41-60%) and poor (0-40%).

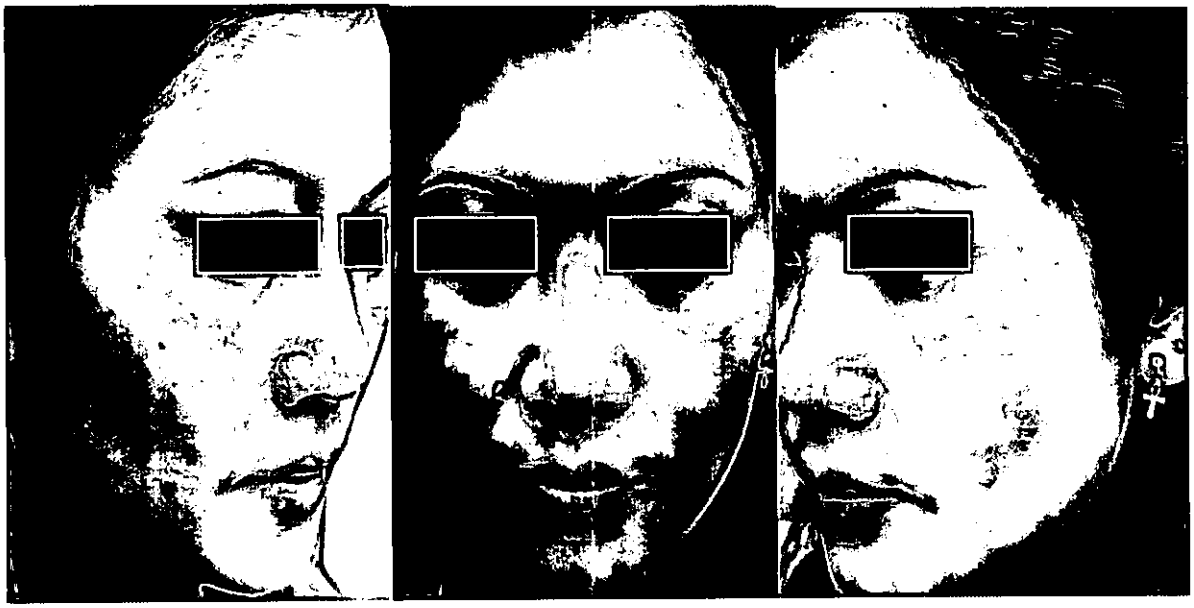
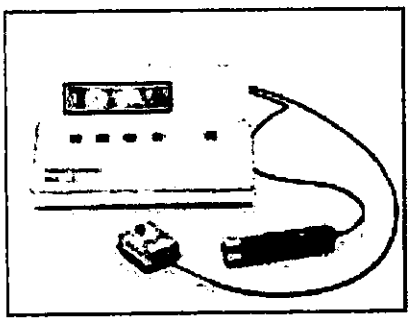


Figure 8 Photographic documentation(right, front, left)

3.3.3. Melanin evaluation by Mexameter



(Mexameter MX 16, 230 V ,50 Hz ,Courage+ Khazaka Gmbh Koln Germany)

The mexameter provides objective measurement of melanin pigment based on the absorption spectra of light and has an accuracy of +,-5%.Mexameter readings range from 1 to 1000 with 1 representing white and 1000 representing black. Mexameter readings are obtained, mean of three readings on both sides of face is recorded at baseline and at weeks 2,4,6,8,and 10.

3.3.4. Clinical evaluation and side effect evaluation

Clinical evaluation was performed by medical team and by use "MASI score". The MASI is an index devised the severity of melasma and changes during therapy. The MASI is calculated based on the area (A) of involvement on a scale of 0 (no involvement) to 6 (90%–100% involvement), the darkness(D) of melasma , on a scale of 0 (absent) to 4 (maximum),and the homogeneity (H) of the hyperpigmentation from 0 (minimal) to 4 (maximum).The score for each side is 0 to 24.

MASI right = 0.15[D(rf)+H(rf)]A(rf)+0.3[D(rm)+H(rm)]A(rm)+0.05[D(rc)+H(rc)]A(rc).

Side effect evaluation was performed by doctor and patients. The doctor recorded the side effect that found during treatment and the patients completed a questionnaire about side effects at the end of treatment as follows :

TABLE 1 Side effects evaluation form.

Adverse effects	Right			Left		
	mild	moderate	severe	mild	moderate	severe
Erythma						
Scaling						
Edema						
Vesicles						
Crusting						
Erosions						

3.3.5.Subjective evaluation.

After treatment at 10 weeks, the patients completed a questionnaire. The improvement was assessed by the patients on a three-point scale as follows: good improvement (more than 61%), fair (41-60%) and poor improvement (0-40%).

TABLE 2 Questionnaire of subjective evaluation.

Patients' assessment H.N.	 Right face Left face	Poor	Fair	Good

3.3.6. Statistical analysis

Statistical analysis by using SPSS program.

MASI score (Melasma Area and Severity Index) : Wilcoxon signed rank test

Melanin index : paired t-test

Global evaluation and side effect : Chi-square test

Chapter 4

Results

Forty two patients were enrolled in the study .All patients are female, the average age of the patients was 38 years (range 21-58 years),the average duration of melasma before study was 4 years (range 1-17 years). All had stopped previous treatments at minimum 4 weeks prior to attending the study. The most common type and pattern of melasma are epidermal and centrofacial respectively. Approximately 23 % of patients had the family history of melasma. Sunlight aggravated melasma in most of the patients. Their demographic data are given in the table 3.

TABLE 3 Demographic data of patients.

Variable	Patients, Number (%) (N=42)
Sex	
Female	42 (100.00%)
Male	0 (0.00%)
Age (years)	
20-30	3 (7.14%)
31-40	17 (40.48%)
41-50	20 (47.62%)
51-60	2 (4.76%)
Duration of melasma	
0-10	38 (90.48%)
11-20	4 (9.52%)
21-30	0 (0.00%)

TABLE 3 Demographic data of patients.(continued)

Fitzpatrick skin types	
III	13 (30.95%)
IV	27 (64.92%)
V	2 (4.76%)
Patterns of melasma	
Centrofacial	25 (59.52%)
Malar	17(40.48%)
Mandibular	0 (0.00%)
Types of melasma by wood lamp examination	
Epidermal	35 (83.33%)
Mixed	7 (16.67%)
Dermal	0 (0.00%)
History of melasma in family	
Yes	10 (23.81%)
No	32 (76.19%)
History of aggravating factors	
Pregnancy	2 (4.76%)
Hormonal therapy	5 (11.90%)
Sun exposure	28 (66.67%)
Cosmetic use	1 (2.38%)
Drugs	0 (0.00%)
Unknown	6 (14.29%)

At the end of the study, thirty eight patients completed the study ,12 from group 1 (HQ vs placebo) , 13 from group 2 (alpha arbutin vs placebo)and 13 from group 3 (HQ vs alpha arbutin). Four patients were excluded from the study because 1 patient developed severe side effect and 3 patients had loss the follow up.

The three groups of patients were evaluated separately about clinical improvement by using melanin index, MASI score , photo evaluation by doctors and subjective evaluation by patients. The results of study present as follow.

4.1 The mean of melanin index

Group 1,the mean of melanin index for 3% hydroquinone group decreased from 513.33 at baseline to 491.16 at the last follow up (10 weeks) .In the control group (the mean of melanin index decreased from 512.66 at baseline to 507.83 at the last follow up.The significant difference (95 % confidence interval) in the mean of melanin index between two groups was also found at week 4,6,8 and 10 (P=0.007,0.001,0.001 and 0.002) Although a significant decrease was seen in both groups, a greater decrease in melanin index was observed in the 3% hydroquinone group.

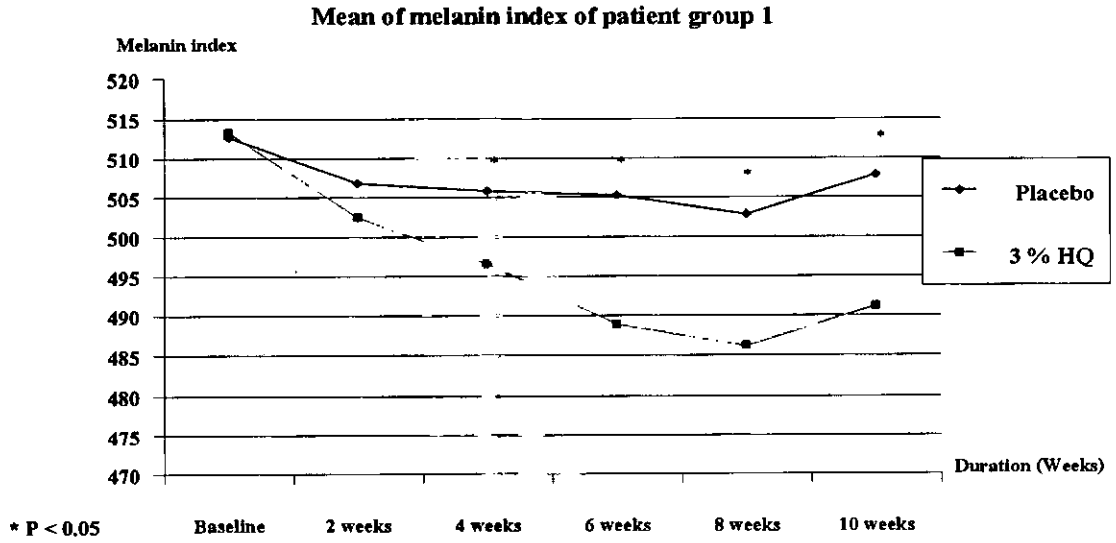


Figure 9 Graph demonstrated the melanin index of group 1

Group 2, the mean of melanin index for 1%alpha arbutin group decreased from 514.15 at baseline to 495.30 at the last follow up (10 weeks). In the control group the mean of melanin index decreased from 514.46 at baseline to 502.23 at the last follow up.The significant difference between two groups was also found only at week 10. (p=0.001) Although a significant decrease was seen in both groups, a greater decrease in melanin index was observed in the 1% alpha arbutin group.

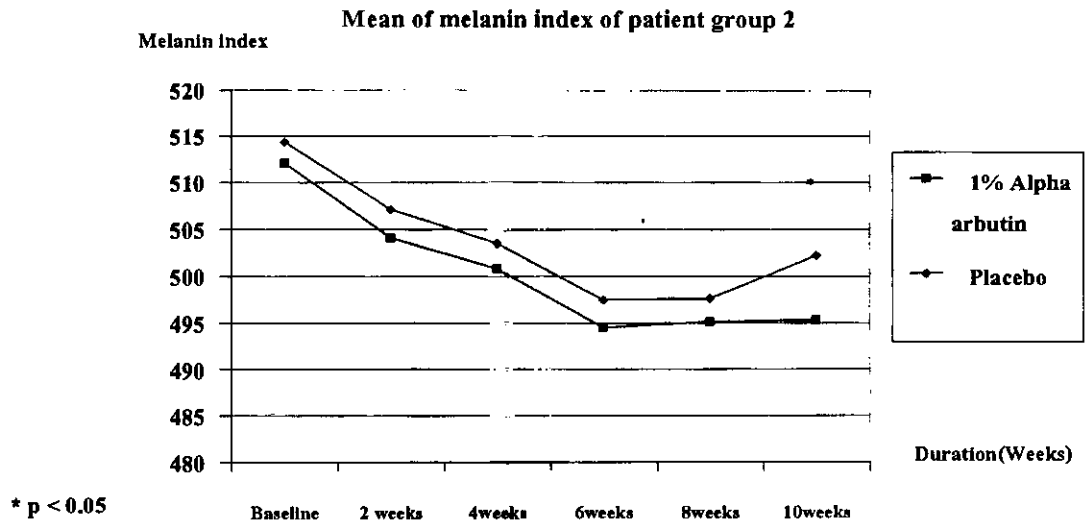


Figure 10 Graph demonstrated the melanin index of group 2

Group 3 , the mean of melanin index for 3% hydroquinone group decreased from 539.69 at baseline to 518.30 at the last follow up (10 weeks) .In the 1% Alpha arbutin group,the mean of melanin index decreased from 537.07 at baseline to 520.69 at the last follow up.But the significant difference between two groups was not found in all of the follow up. Although a significant decrease in melanin index was seen in both groups but a greater decrease in melanin index was observed in the 3% hydroquinone group.

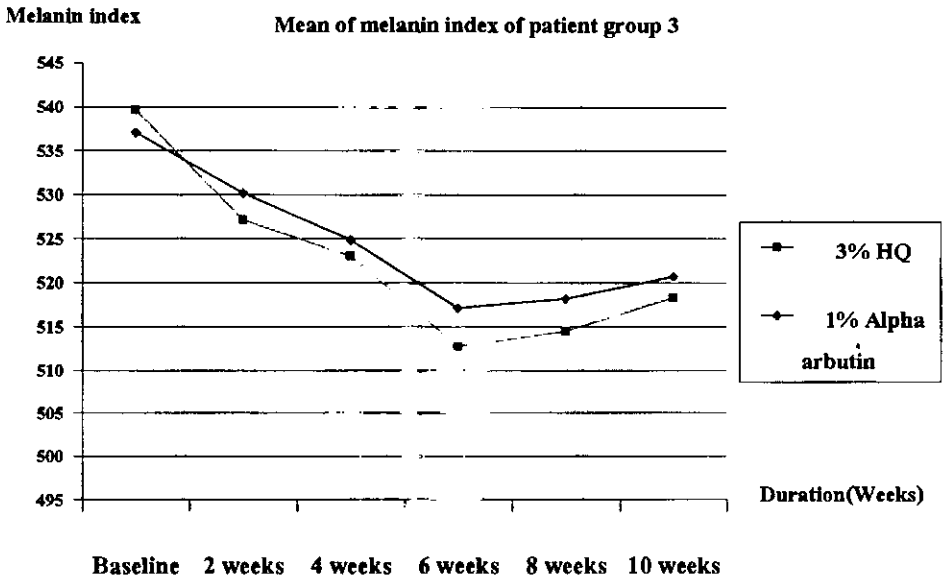


Figure 11 Graph demonstrated the melanin index of group 3

4.2 The mean of MASI score

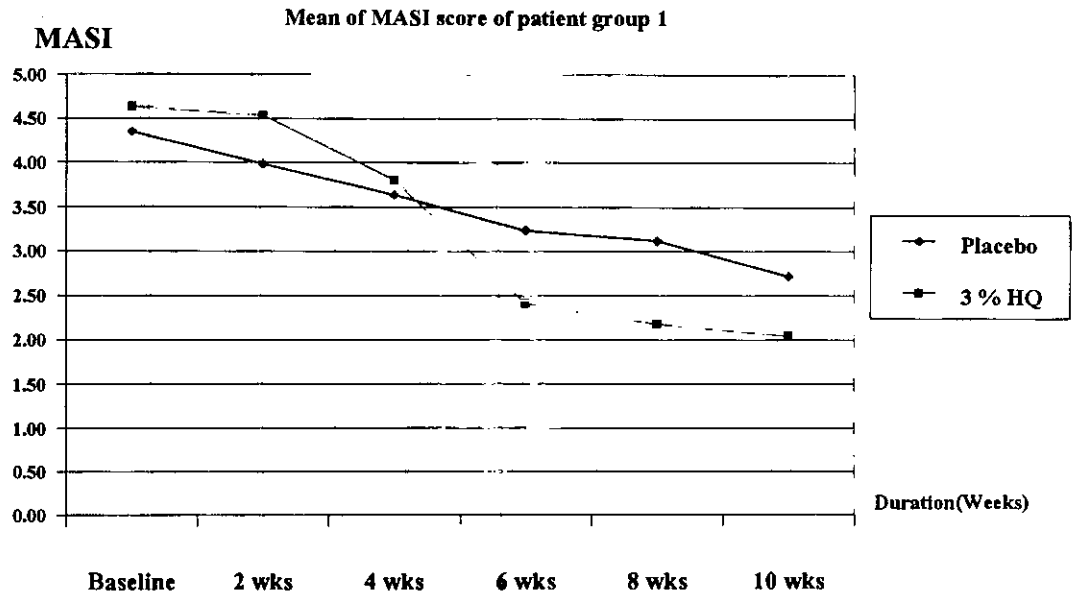


Figure 12 Graph demonstrated the MASI score of group 1

Group 1,the mean of MASI score for 3%hydroquinone group decreased from 4. 63 at baseline to 2.05 at the last follow up (10 weeks) .In the placebo side, the mean of MASI score decreased from 4.35 at baseline to 2.72 at the last follow up. .Although a significant decrease was seen in both sides but a greater clinical lightening was observed in the 3% hydroquinone side. But in this group there is no significant difference in the MASI score between placebo and 3%HQ side.

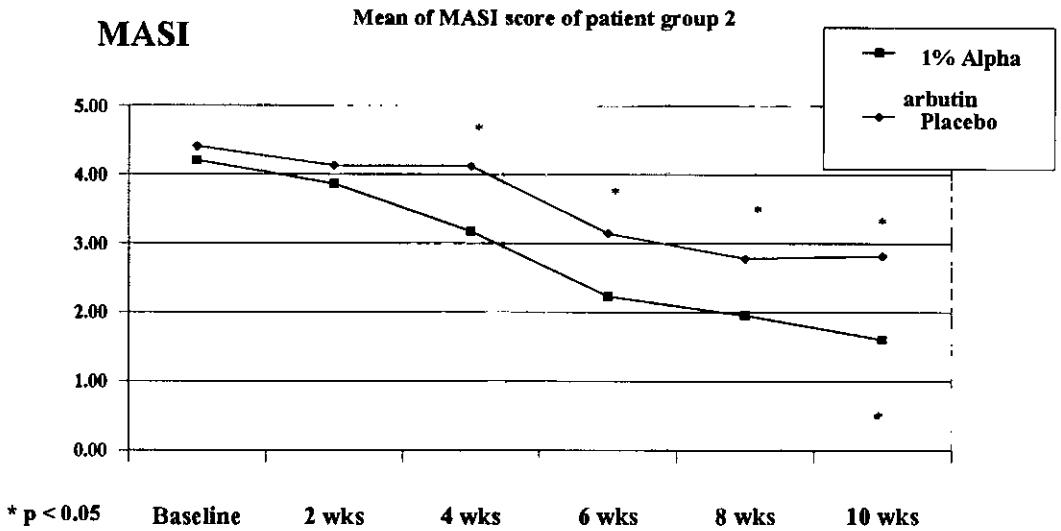


Figure 13 Graph demonstrated the MASI score of group 2

Group 2, the mean of MASI score for 1% alpha arbutin side decreased from 4.28 at baseline to 1.80 at the last follow up (10 weeks) .In the placebo side, the mean of MASI score decreased from 4.41 at baseline to 2.82 at the last follow up. The significant difference between two sides was found at week 4,6,8 and 10 (P=0.01,0.00,0.001 and 0.00)

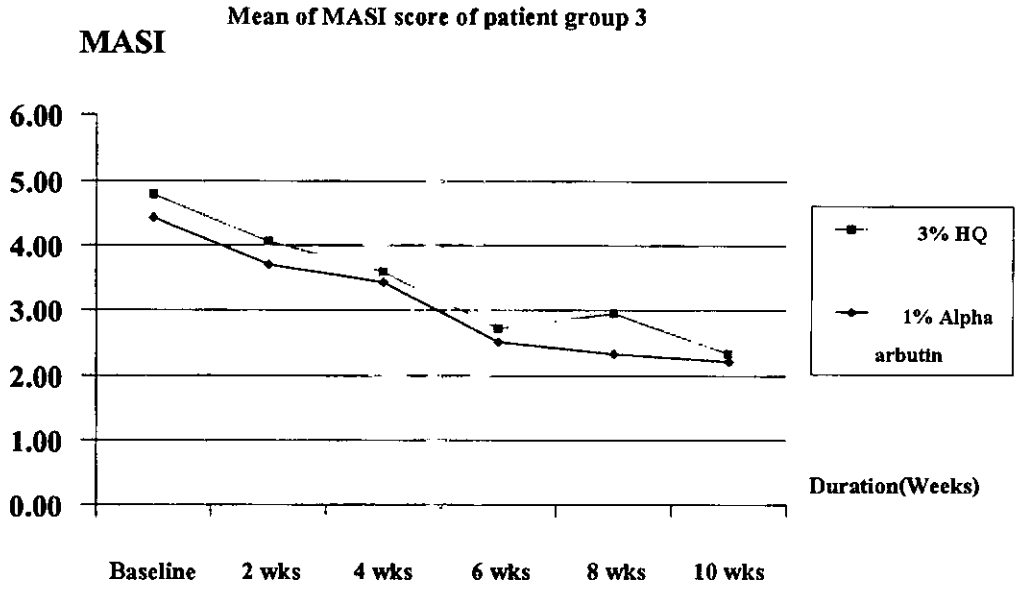


Figure 14 Graph demonstrated the MASI score of group 3

Group 3 , the mean of MASI score for 3% hydroquinone side decreased from 4.78 at baseline to 2.32 at the last follow up (10 weeks) .In the 1% Alpha arbutin side, the mean of MASI score decreased from 4.43 at baseline to 2.21 at the last follow up. There was no significant difference between two sides was found in this group.

4.3 Photo evaluation

The photo evaluation was done at the end of the study by two independent doctors with a three-point scale as follows: good improvement (more than 61%), fair (41-60%) and poor (0-40%).

The results of the photo evaluation showed as follow

TABLE 4 Photo evaluation by doctor 1

		poor	fair	good
Group 1	Placebo	41.67%	33.33%	25.0%
	3%HQ	33.33%	50%	16.67%
Group 2	1% Alpha	15.38%	46.15%	38.46
	Placebo	15.38%	46.15%	38.46%
Group 3	3%HQ	7.69%	69.23%	23.08%
	1% Alpha	15.38%	46.15%	38.46%

TABLE 5 Photo evaluation by doctor 2

		poor	fair	good
Group 1	Placebo	16.67%	50%	33.33%
	3%HQ	8.33%	8.33%	83.33%
Group 2	1% Alpha	23.08%	38.46%	38.46%
	Placebo	30.77%	30.77%	38.46%
Group 3	3%HQ	15.38%	23.08%	61.54%
	1% Alpha	7.69%	38.46%	53.84%

The results of the photo evaluation by two doctors showed no significant different in the most of patients. The significant different in the photo evaluation was found only in Doctor 2 's evaluation in patients group 1(placebo and 3%HQ) .($p=0.012$)

Figure 15 Show the photographs of a patient with clinical improvement after applying 3% hydroquinone cream to the right half of face .

4.4 Patient's own assessment

The evaluation at 10 weeks was done by patient's own assessment, improvement was assessed on a three -point scale as follows: good improvement (more than 61%), fair (41-60%) and poor (0-40%)

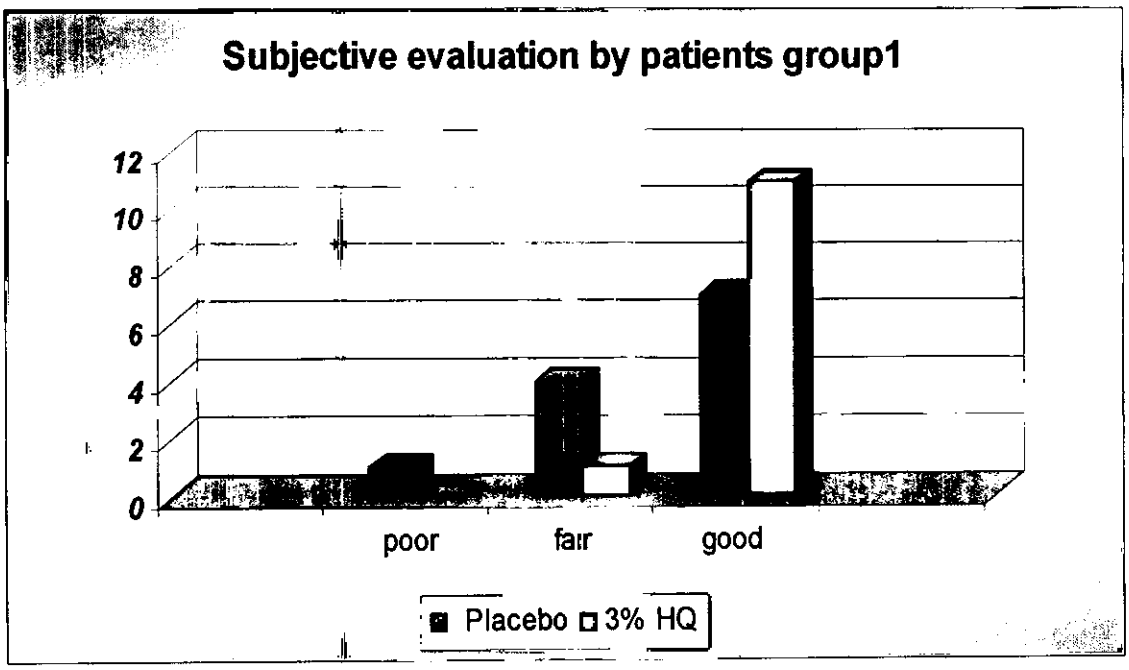


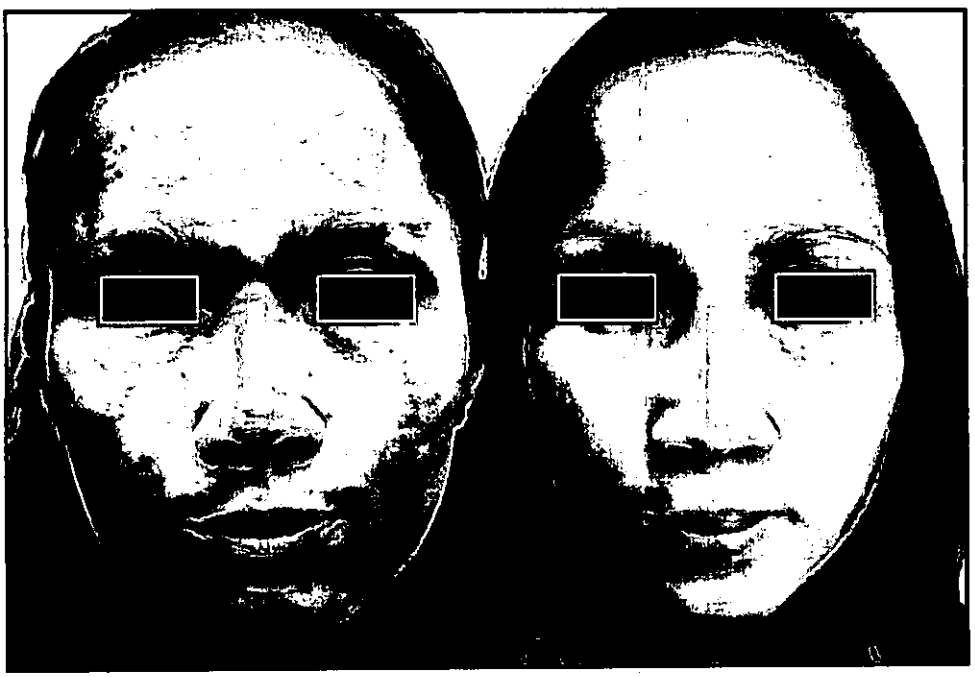
Figure 18 Graph demonstrated the subjective evaluation of group 1

The patients in group1 graded their improvement on the placebo side: 58.33% as good, 33.33 % as fair and 8.33 % as poor .For the 3%HQ side, the patients graded their improvement on this side of face as good 91.67 %, 8.33 % as fair and 0% as poor.

The mean of improvement score in this group shows as follow: Placebo = 3.583 and 3%HQ = 4.416 and there was statistically significant difference between placebo and 3%HQ (P = .005)

These results showed that the patients of group 1 satisfied with the treatment of 3% HQ more than placebo.

Figure 16 Show the photographs of a patient with clinical improvement after applying 1 % alpha arbutin cream to the left half of face .



Figures 17 Show the photographs of a patient with clinical improvement after applying 3% hydroquinone cream to the right half of the face and 1%alpha arbutin to the left half of the face.

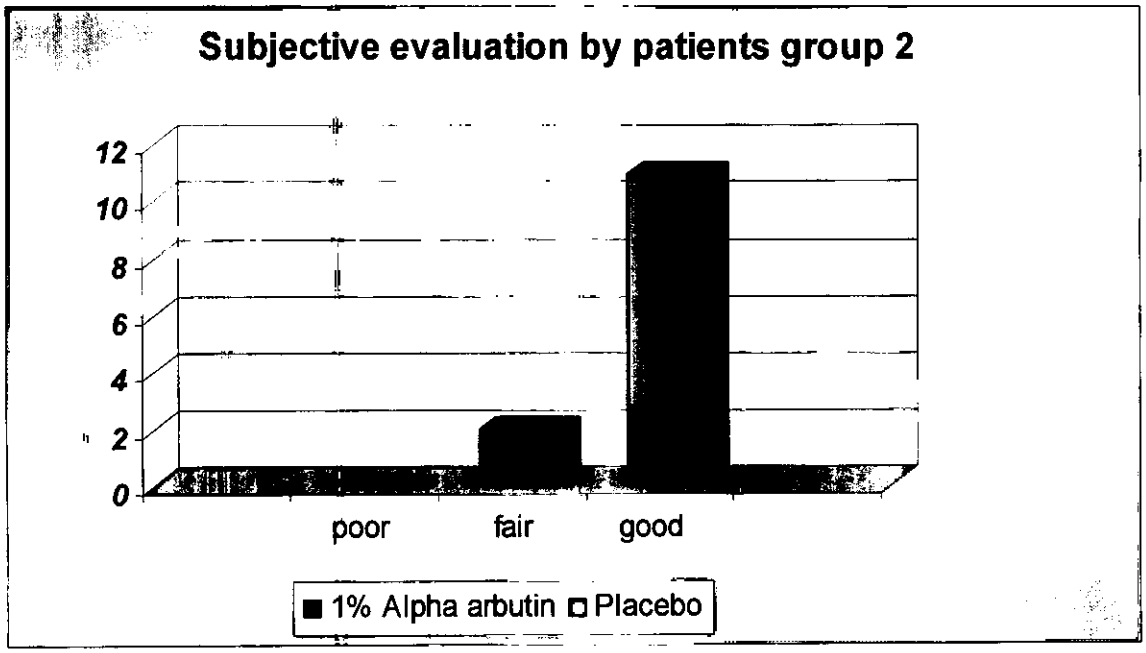


Figure 19 Graph demonstrated the subjective evaluation of group 2

Group 2, 84.62% of patients graded their improvement on 1% alpha arbutin side as good, 15.38 % as fair and 0 % as poor. For the placebo side, 84.62 % of patients graded their improvement as good, 15.38 % as fair and 0% as poor.

The mean of improvement score in this group shows as follow: 1%alpha arbutin = 4.0769 and placebo = 3.8462 but there was no statistically significant difference between using 1%alpha arbutin and using placebo in this group.

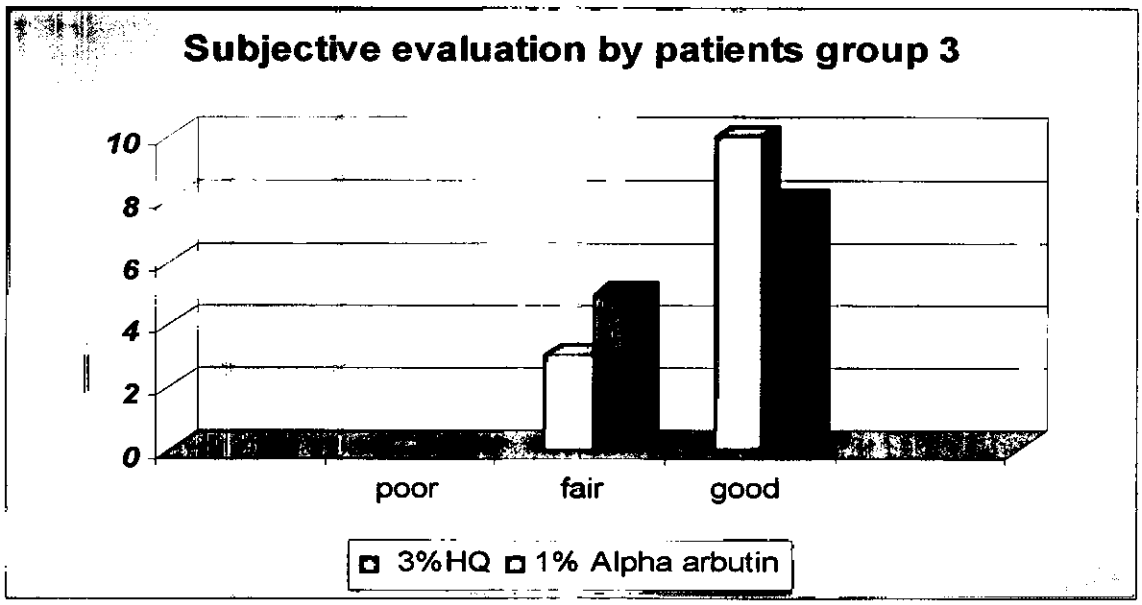


Figure 20 Graph demonstrated the subjective evaluation of group 3

Group 3, 76.92 % of patients graded their improvement on the 3% HQ side as good, 23.08 % as fair and 0% as poor .For the 1% alpha arbutin side, 61.60 % of patients graded their improvement as good, 38.40 % as fair and 0% as poor .The mean of improvement score in this group shows as follow: 3%HQ = 4.076 and 1% alpha arbutin = 3.769 but there was no statistically significant difference between using 3%HQ and using 1% alpha arbutin in this group.

TABLE 6 Subjective evaluation by patients

		poor	fair	good
Group 1	Placebo	8.33%	33.33%	58.33%
	3%HQ	0%	8.33%	91.67%
Group 2	1% Alpha	0%	15.38%	84.62%
	Placebo	0%	15.38%	84.62%
Group 3	3%HQ	0%	23.08%	76.92%
	1% Alpha	0%	38.40%	61.60%

4.5 The side effects.

The incidence of side-effects was greater among the patients in group 1, with 4 cases of patients reporting the mild, moderate and severe side effect, 1 cases developed mild side effect on placebo side ,3 cases developed on 3%HQ side, One patients developed severe redness and irritation from using 3% hydroquinone and she was terminated from the study at the 4^{th} week.

Group 2, there were no side effect from the cream (1% Alpha arbutin and placebo) that use in the study but there was irritation in one patient who use sunscreen , nevertheless this patient could continue and complete the study.

Group 3, there were 2 cases complaints of mild side-effects, 1 case on the 3%HQ side ,1cases on 1% alpha arbutin side .No patient in this group was terminated from the study.

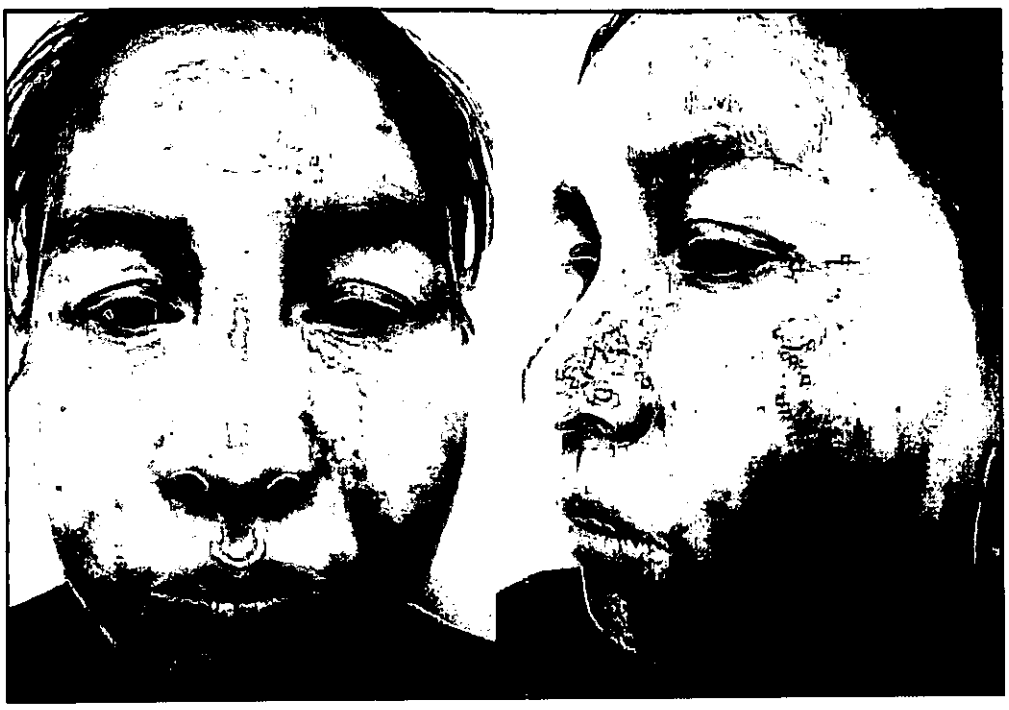


Figure 21 The side effect from 3% Hydroquinone on left face of patient.

CHAPTER 5

DISCUSSIONS

5.1 Demographic data of the patients.

Melasma is a circumscribed hyperpigmentation of the facial skin over sun-exposed areas; it is commonly seen in Asian women. The disease affects all racial groups but is most prevalent in darker individuals (skin types IV, V, and VI). [47]. It is exacerbated by sun exposure, pregnancy, oral contraceptives, and certain anti-epilepsy drugs.

According to our studying in patients' demographic data, the majority was the middle age women , their average age was 38 years , Fitzpatrick skin type IV (65%) , centrofacial pattern (60%) , epidermal type (80%) , the average duration of melasma was 4 years and the aggravating factor was mostly from sun exposure (66%). This data shown is similar to the other previous studies, for examples : the study of Ian L.Guevara et al : Safety and efficacy of 4% hydroquinone combined with 10% glycolic acid in the treatment of melasma[53] showed their patients' demographic data that melasma was commonly seen in the people with IV skin types.(61%), the average age was 37 years, the average duration was 9 years , the most common pattern was centrofacial (80%), aggravating factor was mostly from sun exposure (63%). Besides, there are also two other studies of Pathak MA, Farinelli WA, Fitzpatrick T: Nature of melanin pigmentation induced by solar or UV radiation in individuals of skin types I to VI. [54]. and Gilchrest BA, Eller MS : DNA photodamage stimulates melanogenesis and other photoprotective responses. [55] .They showed that the average age of the patients was 34 years, the most common type of melasma was epidermal type(70-78%) and the most important predisposing factor for melasma was the sun exposure (60-65%).

5.2 The efficacy of alpha arbutin in the treatment of melasma.

Melasma still has a therapeutic problem with enormous variety of treatments, which range from the isolated use of hypopigmenting agents such as hydroquinone improves between 64 - 88% [6,7] , 68% improvement for retinoic acid [48] , 75% through combinations of the Kligman triad [49], chemical peels [50] and including unsuccessful attempts with lasers, such as the Q-switched ruby equipment. [51]

It is known that hydroquinone is one of the effective depigmenting agent in the treatment of melasma. Because of the serious side effect in the cases with long-term treatment by hydroquinone i.e. orchonosis and permanent leukoderma, it should always be treated with caution. Adverse effects related to hydroquinone frequently occur with the formulations of > 4%. The most common problems caused by hydroquinone are irritant and allergic contact dermatitis. [52].

Alpha arbutin (4-hydroxyphenyl alpha-glucopyranoside) is known as the new derivative of hydroquinone. It is the effective depigmenting agent in the vitro study but the clinical study is lacking. Sugimoto K et al had previously studied the inhibitory effects of 4-hydroxyphenyl alpha-glucopyranoside (alpha-arbutin) on melanogenesis in cultured human melanoma cells, HMV-II, and in a three-dimensional cultured human skin model . Their study shows that alpha-arbutin do not inhibit cell viability, while melanin synthesis is reduced to 40% of that in the control. These results indicate that alpha arbutin is an effective and safe ingredient for skin-lightening. [19]

To assess efficacy and safety of alpha arbutin for melasma treatment then we performed this clinical study. The results reveal that 1% alpha arbutin is an effective depigmenting agent for pigmentation reduction in melasma with minimal side effect . A greater clinical lightening (MASI score) was observed in the 1% alpha arbutin group more than placebo with statistical significant different at 4^{th} week ($p=0.01$) and the difference in pigment reduction (melanin index) between using of 1% alpha arbutin against placebo was statistically significant at 10^{th} week ($p=0.001$)

Comparing 1% alpha arbutin with 3% hydroquinone cream, our study shows indifferent in efficacy (MASI and Melanin index), while the onset of improvement in pigment reduction (melanin index) of 3% hydroquinone is faster than 1% alpha arbutin. The significant different in pigment reduction of hydroquinone was observed as early as the 4 th week of treatment, whereas that of alpha arbutin was noted at the10 th weeks.

Interesting, between the 8 th to 10th week of the study, it had been remarkably found that the melanin index observed in the most of patients showed increasing. We try to explain the cause of increasing of the melanin index. It was the actual period that patients went on sunlight vacation in April, some went to the beach, traveled to their hometown and cerebrated Songkran festival, which might affect the increasing of melanin index.

5.3 The side effects of alpha arbutin.

The side effects of 1% alpha arbutin, comparing to 3% hydroquinone, has been found to be less irritating and better tolerated. Four cases (14.2%) of the patients in hydroquinone group developed mild to moderate erythema with 3% hydroquinone cream at the first follow up visit , whereas 3 cases were able to continue the trial with the addition of a moisturizer applied after 3%hydroquinone cream application. Only one patient in hydroquinone group stopped using of 3% hydroquinone cream because of severe irritation, then necessary to be cut out of the study. Still in the alpha arbutin group, it was found only one patient (3.5%) who developed mild erythema and scaling at the first follow up and be able to continue and complete the study. Such the results show that alpha-arbutin is an effective and safe ingredient for skin-lightening in melasma treatment. Nevertheless the long term side effects from the using of alpha arbutin should be observed in the further longer follow-up study.

5.4 Assessment by patients and doctors.

According to the patients' own assessment, the patients have been more satisfied with the treatment of 3% hydroquinone cream than placebo with statistically significant (P = 0.005). The subjective improvement was observed on the hydroquinone side with 91.67% good results, compared with 58.33% on the placebo side, despite of no difference with 1% alpha arbutin and placebo. Besides, between 3% hydroquinone and 1% alpha arbutin, they also felt it made no difference in the treatment of their melasma.

According to the evaluation by two independent doctors, the significant different in photo evaluation was found only in Doctor 2 's evaluation which showed that the photo of the face using hydroquinone had the better improvement than using placebo (p=0.012). But the majority's assessments by two doctors showed that the clinical improvement by photo evaluation in the treatment of melasma, the using of hydroquinone, alpha arbutin and placebo are not different.

5.5 Conclusion.

From our study, we can conclude that 1% alpha arbutin is an effective depigmenting agent and it can reduce the pigmentation as effective as 3% HQ in the treatment of melasma, with a minimum of adverse effects. For this reason, 1% alpha arbutin seems to be a new interesting choice for the treatment of melasma, however, further trials are required to assess the efficacy and safety of the higher concentration of alpha arbutin or the combination of this topical drug with the others, in order to identify additive effects for better treatments of melasma. Nevertheless, the drawbacks of the study are the number of the patients should be larger with a longer follow-up period.

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Appendix

Appendix

<u>แบบบันทึกข้อมูลโครงการวิจัย</u>

เรื่อง การเปรียบเทียบประสิทธิภาพของสาร1%อัลฟาอาบูติน

กับสาร 3%ไฮโดรควิโนน ในการรักษาฝ้า

เลขที่แบบบันทึกข้อมูล.....

ข้อมูลทั่วไปของผู้ป่วย (patient demographic information)

เฉพาะเจ้าหน้าที่

1. วัน/ เดือน/ปี ที่เก็บข้อมูล..... Date
2. ชื่อ/ นามสกุล..... Name
3. Hospital Number ..... HN
4. บ้านเลขที่..... Address

   เบอร์โทรศัพท์..... Tel.
5. เพศ .....1. ชาย .....2. หญิง Sex
6. อายุ.....ปี Age
7. อาชีพ .....1.ข้าราชการ .....2. พนักงาน Occupation

   .....3. แม่บ้าน .....4. นักเรียน/นักศึกษา

   .....5. กิจการส่วนตัว .....6. อื่นๆ
8. ระยะเวลาที่เป็นฝ้า (yr) Duration
9. ประวัติคนในครอบครัวเป็นฝ้า ....1. มี ...2. ไม่มี FH
10. ปัจจัยกระตุ้นที่ทำให้เกิดฝ้า Aggravating

    ....1. ตั้งครรภ์ ....2. การได้รับฮอร์โมน

    ....3. การได้รับแสงแดด ....4. การใช้เครื่องสำอาง

    ....5. ยากันชัก ....6. ยาที่มีปฏิกิริยากับแสง
11. ประวัติการรักษาที่เคยได้รับมาก่อน

    ....1. เคย ....2. ไม่เคย
12. ชนิดของฝ้าจำแนกด้วยการตรวจ wood's lamp wood's lamp

    ....1.epidermal type

    ....2. mixed (epidermal-dermal) type

13. จำแนกชนิดของฝ้าตามบริเวณที่เป็น pattern

....1. centrofacial type ....2. malar type

....3. mandibular type

14. จำแนกชนิดของสีผิวตาม skin photo type ....2. , ...3. , ...4. , ...5. Skin type

<u>บันทึกผลการทดลอง</u>

การประเมินประสิทธิภาพการรักษา

1. MASI score Rt = ... Lt =.....

Melasma area (A)

%area involvement	right			left		
	Frontal (f) 15%	Malar (m) 30%	Chin (c) 5%	Frontal (f) 15%	Malar(m) 30%	Chin(c) 5%
no involvement = o						
0%-9% = 1						
10%-29% = 2						
30%-49% = 3						
50%-69% = 4						

70%-89% = 5						
90%-100% = 6						

Severity index

Homogeneity (H)

Homogeneity (H)	right			left		
	Frontal (f)	Malar (m)	Chin (c)	Frontal (f)	Malar(m)	Chin(c)
Absent = 0						
Slight = 1						
Mild = 2						
Marked = 3						
Maximum/ severe =4						

Darkness (D)

Darkness (D)	right			left		
	Frontal (f)	Malar (m)	Chin (c)	Frontal (f)	Malar(m)	Chin(c)
Absent = 0						
Slight = 1						
Mild = 2						
Marked = 3						
Maximum/ severe =4						

2. melanin index (MI) ..... (by mexameter)

Right malar			Left malar		

แบบฟอร์มประเมินผลการรักษาโดยรวม (global evaluation) :

- โดยผู้ป่วย

ความพึงพอใจจากผลการรักษาโดยรวม	น้อยมาก (1) very poor 0-20%	ค่อนข้างน้อย (2) Poor 21-40%	ปานกลาง (3) fair 41-60%	ค่อนข้างมาก (4) good 61-80%	มากที่สุด (5) excellent >81%
หน้าด้านขวา					
หน้าด้านซ้าย					

- โดยแพทย์

ความพึงพอใจจากผลการรักษาโดยรวม	น้อยมาก (1) very poor 0-20%	ค่อนข้างน้อย (2) Poor 21-40%	ปานกลาง (3) fair 41-60%	ค่อนข้างมาก (4) good 61-80%	มากที่สุด (5) excellent >81%
หน้าด้านขวา					
หน้าด้านซ้าย					

การประเมินผลข้างเคียงจากการใช้ยา : โดยผู้ป่วย ....1. มี ...2. ไม่มี Adverse effects

ผลข้างเคียง	ไม่มีเลย (0)	น้อยมาก (1)	ค่อนข้างน้อย (2)	ปานกลาง (3)	ค่อนข้างมาก (4)	มากที่สุด (5)
แสบร้อน						
คัน						
แดง						
แห้ง						
ลอก						

โดยแพทย์ (Physician evaluation)

Adverse effects	right			left		
	mild	moderate	severe	mild	moderate	severe
Erythma						
Scaling						
Edema						
Vesicles						
Crusting						
Erosions						

ข้อมูลสำหรับผู้ป่วย

เรียน ผู้ป่วยทุกท่าน

ท่านเป็นผู้ได้รับเชิญจากแพทย์ให้เข้าร่วมการศึกษาทางคลินิกเพื่อเปรียบเทียบประสิทธิภาพของสาร 1 % อัลฟาอาบูติน กับสาร 3%ไฮโดรควิโนน ในการรักษาฝ้า ก่อนที่ท่านตกลงเข้าร่วมการศึกษาดังกล่าว ขอเรียนให้ท่านทราบถึงเหตุผลและรายละเอียดของการศึกษาวิจัยครั้งนี้

ชื่อโครงการ : การศึกษาทางคลินิกเพื่อเปรียบเทียบประสิทธิภาพของสาร 1% อัลฟาอาบูติน กับสาร3% ไฮโดรควิโนน ในการรักษาฝ้า

สถานที่ทำการวิจัย : ศูนย์ผิวหนัง คณะแพทยศาสตร์ มหาวิทยาลัยศรีนครินทร์วิโรฒประสานมิตร

ผู้รับผิดชอบโครงการวิจัย

1. นพ.ธีรศักดิ์ หมอยาดี
2. รศ.นพ. มนตรี อุดมเพทายกุล
3. ผศ.พญ. สุวิรากร โอภาสวงศ์
4. รศ.นพ. ปิติ พลังวชิรา

วัตถุประสงค์การวิจัย : เพื่อประเมินประสิทธิภาพของสารเพื่อให้ผิวขาว1% อัลฟาอาบูตินในการรักษาฝ้าที่หน้า

ประโยชน์ของโครงการวิจัย

1. เพื่อประเมินประสิทธิภาพและความปลอดภัยของสารเพื่อให้ผิวขาวชนิดใหม่ 1% อัลฟาอาบูตินในการใช้รักษาฝ้าที่หน้า
2. ผลการวิจัยสามารถนำไปสู่การตัดสินใจของแพทย์ในการเลือกใช้ยารักษาฝ้าที่ได้ผลดีและมีผลข้างเคียงน้อย

เกณฑ์ในการคัดเลือกผู้ป่วย

1. ผู้ป่วยหญิงไทย อายุระหว่าง 20-60 ปี มีปัญหาฝ้าที่หน้า
2. ผู้ป่วยสามารถมาพบแพทย์ตามนัดที่สัปดาห์ที่ 2,4,6,8,10 และ 12 ได้
3. ผู้ป่วยสามารถยอมรับเงื่อนไขต่างๆที่โครงการกำหนด และสามารถปฏิบัติตามได้อย่างเคร่งครัด และสามารถลงลายมือชื่อในหนังสือให้ความยินยอมก่อนเข้าร่วมโครงการวิจัย
4. ผู้ป่วยต้องไม่เป็นผู้ที่มีโรคผิวหนังที่กำลังอยู่ในระยะเฉียบพลัน
5. ผู้ป่วยต้องไม่เป็นผู้ที่มีประวัติแพ้สารสำคัญที่เป็นส่วนประกอบของผลิตภัณฑ์ที่ใช้ในการวิจัย
6. ผู้ป่วยต้องไม่เป็นผู้ที่ได้รับการรักษาฝ้ามาภายในระยะเวลา 1เดือนก่อนเข้าร่วมโครงการ

7. ผู้ป่วยต้องไม่เป็นผู้ที่อยู่ในระหว่างการได้รับยาที่มีผลต่อการรักษาฝ้าเช่น กรดวิตามินเอ ยาคุมกำเนิดเป็นต้น

8. ผู้ป่วยต้องไม่เป็นผู้ที่อยู่ในระหว่างตั้งครรภ์หรือให้นมบุตร หรือมีโรคประจำตัวเกี่ยวกับความผิดปกติของฮอร์โมน

วิธีการศึกษา

โรคฝ้า(Melasma) เป็นโรคที่เกิดจากความผิดปกติของสีผิว ทำให้สีผิวเข้มขึ้นกว่าปกติเป็นรอยปื้นสีน้ำตาลหรือน้ำตาลเข้ม พบได้บ่อยบริเวณใบหน้าในผู้หญิงวัยกลางคน การรักษาที่ได้ผลดีวิธีหนึ่งก็คือการใช้สารเพื่อให้ผิวขาวได้แก่ สารอัลฟาอาบูติน และสารไฮโดรควิโนน ในรูปแบบยาทา

โดยท่านจะได้รับยาทาฝ้าที่หน้า ท่านละ 2 หลอดโดยท่านจะต้องทายาดังกล่าวให้ทั่วบนใบหน้าแต่ละด้าน ซ้าย- ขวาตามที่ฉลากยาข้างหลอดระบุ โดยทาก่อนนอนทุกวันเป็นระยะเวลา 3 เดือน

นอกจากนี้ท่านจะได้รับสารกันแดดชนิดออกฤทธิ์ป้องกันกว้าง (sunscreen SPF 30)สำหรับทาตอนเช้าก่อนออกจากบ้านและโฟมสำหรับทำความสะอาดผิวหน้าคุณภาพดีสำหรับใช้ทำความสะอาดผิวหน้า

โดยตัวยาทั้งสองชนิดนี้มีการศึกษาทดลองมาก่อนหน้าแล้วว่าได้ผลในการรักษาฝ้าได้ดี โดยไม่มีผลข้างเคียงที่เป็นอันตรายร้ายแรงแต่อย่างใด หากท่านมีข้อสงสัยเกี่ยวกับวิธีการศึกษาวิจัย แพทย์ผู้รับผิดชอบโครงการวิจัยยินดีตอบคำถามต่าง ๆ ที่ท่านสงสัยโดยละเอียด

หากท่านตกลงที่จะเข้าร่วมการศึกษาวิจัยนี้ จะมีข้อปฏิบัติร่วมดังต่อไปนี้

1. ท่านจะได้รับยาทาฝ้าจำนวน 2 หลอด ครีมกันแดดและโฟมล้างหน้า และท่านจะต้องกลับมาพบแพทย์ตามนัด โดยท่านจะไม่เสียค่าใช้จ่ายใด ๆทั้งสิ้นตลอดการวิจัย

2. กรณีเกิดผลข้างเคียงจากการวิจัยผู้เข้าร่วมโครงการจะได้รับการรักษาโดยแพทย์ผู้เชี่ยวชาญโดยไม่เสียค่าใช้จ่ายใดๆทั้งสิ้นจนกว่าผลข้างเคียงจะดีขึ้น

3. ในกรณีที่ท่านเข้าร่วมโครงการวิจัยจนครบระยะเวลาที่กำหนดแต่ฝ้ายังไม่ดีขึ้นหรือแย่ลง จะได้รับการพิจารณาจากแพทย์ในการให้ผลิตภัณฑ์รักษาฝ้าที่เหมาะสมเป็นรายๆไป.

4.หลังให้การรักษาแล้ว แพทย์จะให้ใบประเมินผลการรักษากับท่าน ท่านกรุณากรอกใบประเมิน เพื่อแพทย์ผู้ทำการวิจัยจะได้นำไปใช้ในการวิเคราะห์ข้อมูลต่อไป

5. ข้อมูลต่างๆของท่านจะถูกเก็บเป็นความลับ และจะเปิดเผยเฉพาะข้อมูลที่ได้สรุปผลหลังเสร็จสิ้นโครงการวิจัยแล้วเท่านั้น

6. การเข้าร่วมการศึกษานี้เป็นไปโดยสมัครใจ ท่านอาจจะปฏิเสธที่จะเข้าร่วมหรือถอนตัวจากการศึกษานี้ได้ทุกเมื่อ โดยจะไม่กระทบต่อการดูแลรักษาที่ท่านจะได้รับจากแพทย์

ขอขอบคุณในความร่วมมือของท่านมา ณ ที่นี้

หนังสือให้ความยินยอมของผู้เข้าร่วมโครงการวิจัย

เขียนที่
วันที่

ข้าพเจ้า ชื่อ ....นามสกุล.....อายุ.....ปี

ที่อยู่.....

เบอร์โทรศัพท์ .....

ขอทำหนังสือนี้ให้ไว้ต่อหัวหน้าโครงการวิจัยเพื่อเป็นหลักฐานแสดงว่า

ข้าพเจ้าได้รับทราบจากแพทย์ผู้รับผิดชอบโครงการวิจัยคือ นายแพทย์ธีรศักดิ์ หมอยาดี ซึ่งได้ลงนามด้านท้ายของหนังสือนี้ ถึงวัตถุประสงค์ ลักษณะและแนวทางการศึกษาโครงการวิจัย เรื่อง การศึกษาในระดับคลินิกเพื่อเปรียบเทียบประสิทธิภาพของสารเพื่อให้ผิวขาว1% อัลฟ่าอาบูดินกับสาร3%ไฮโดรควิโนนในการรักษาฝ้า รวมทั้งทราบถึงผลดี ผลข้างเคียง และความเสี่ยงที่อาจเกิดขึ้น ข้าพเจ้าได้ซักถาม ทำความเข้าใจเกี่ยวกับการศึกษาดังกล่าวนี้เป็นที่เรียบร้อยแล้ว

ข้าพเจ้ายินดีเข้าร่วมการศึกษาวิจัยครั้งนี้โดยสมัครใจ โดยมิได้มีการบังคับขู่เข็ญ และอาจถอนตัวจากการเข้าร่วมศึกษานี้เมื่อใดก็ได้ และยอมรับสิ่งไม่พึงประสงค์ที่อาจเกิดขึ้น และจะปฏิบัติตัวตามคำแนะนำของแพทย์ทุกประการ

ข้าพเจ้าได้รับทราบจากแพทย์ผู้รักษาว่า หากข้าพเจ้าได้รับความผิดปกติและอันตรายใดๆอันเกิดขึ้นเนื่องจากการวิจัยดังกล่าว ข้าพเจ้าจะได้รับความคุ้มครองตามกฎหมาย และจะได้รับการรักษาจากคณะผู้วิจัย โดยไม่เสียค่าใช้จ่ายและจะได้รับค่าชดเชยรายได้ที่สูญเสียไปในระหว่างการรักษาพยาบาลดังกล่าว ตลอดจนมีสิทธิ์ได้รับค่าทดแทนความพิการที่อาจเกิดขึ้นจาการวิจัยตามสมควร แต่หากข้าพเจ้าเข้ารับการรักษาทางการแพทย์อื่น ๆ โดยมิได้ปรึกษาแพทย์ผู้รับผิดชอบการศึกษานี้ และมิได้แจ้งให้แพทย์ทราบในทันทีถึงความผิดปกติของร่างกายที่ได้เกิดขึ้น จะถือว่าข้าพเจ้าทำให้การคุ้มครองความปลอดภัยเป็นโมฆะ (ตามที่กฎหมายกำหนด)

ข้าพเจ้ายินดีให้ข้อมูลของข้าพเจ้าแก่คณะแพทย์ผู้รักษา เพื่อเป็นประโยชน์ในการศึกษาวิจัยครั้งนี้ และข้าพเจ้าได้รับการรับรองจากแพทย์ผู้ทำการวิจัยว่าจะเก็บข้อมูลส่วนตัวของข้าพเจ้าเป็นความลับ และจะเปิดเผยเฉพาะสรุปผลการวิจัยเท่านั้น

ข้าพเจ้าได้อ่านและเข้าใจข้อความหนังสือนี้โดยตลอดแล้วและยินดีเข้าร่วมการศึกษานี้ภายใต้เงื่อนไขที่ได้ระบุไว้แล้วในข้างต้น จึงได้ลงลายมือชื่อไว้พร้อมกับหัวหน้าโครงการวิจัยและพยาน

ลงชื่อ.....

( )

นามผู้ป่วย/ผู้ยินยอม

ลงชื่อ.....

( )

ลงนามแพทย์ผู้ให้การรักษา/หัวหน้าโครงการวิจัย

ลงชื่อ.....

( )

พยาน

ลงชื่อ.....

( )

พยาน

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A CLINICAL, PROSPECTIVE, RANDOMIZED, DOUBLE-BLIND TRIAL COMPARING 1% ALPHA ARBUTIN WITH 3% HYDROQUINONE IN THE TREATMENT OF MELASMA.

AN ABSTRACT

BY

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Teerasak Moryadee. (2005). *A clinical, prospective, randomized, double-blind trial, comparing 1 % Alpha arbutin with 3% hydroquinone in the treatment of melasma.* Master thesis, M.Sc. (Dermatology). Bangkok: Graduate School, Srinakharinwirot University. Advisor Committee : Assoc. Prof. Montree Udompatikul, Assoc. Prof. Piti Palungwachira.

Melasma is an acquired hyperpigmentation that is often recalcitrant to treatment with depigmenting agents. Currently hydroquinone is known as one of the most popular depigmenting agent but in the long-term treatment, it had the serious side effects . Recently, alpha arbutin, a new derivative of hydroquinone which is effective and safe approach for skin lightening in the vitro study. Despite of lacking of clinical study, we performed the clinical study to assess the efficacy and safety of using alpha arbutin in the treatment of melasma comparing with hydroquinone and placebo. Forty two Thai women with melasma were enrolled in a split-face, prospective, double-blind, randomized trial lasting for 10 weeks. Pigmentation was measured by using the mexameter. Clinical evaluation and adverse effects were performed by physicians and patients. At the end of the study, the results showed that the treatment of melasma with 1% alpha arbutin cream and 3% hydroquinone cream , both had a significant effect in reducing skin pigmentation comparing with placebo(P=0.007and P=0.001). Comparing 1% alpha arbutin against 3% hydroquinone cream, the results showed no different in efficacy. The side effects of 1% alpha arbutin (4.5%) , comparing with 3% hydroquinone(14.2%) , was found to be less irritating and better tolerated . From our study, we can conclude that 1% alpha arbutin can reduce the pigmentation as effective as 3% hydroquinone in the treatment of melasma, with a minimum of adverse effects. For this reason, 1% alpha arbutin seems to be a new interesting choice for the treatment of melasma.

การศึกษาทางคลินิกเปรียบเทียบประสิทธิภาพการใช้สารเพื่อให้ผิวขาว 1 % Alpha arbutin กับ 3% Hydroquinone ในการรักษาฝ้าที่หน้า

บทคัดย่อ

ของ

นายแพทย์ธีรศักดิ์ หมอยาดี

เสนอต่อบัณฑิตวิทยาลัย มหาวิทยาลัยศรีนครินทรวิโรฒ เพื่อเป็นส่วนหนึ่งของการศึกษา

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พฤษภาคม 2548

ธีรศักดิ์ หมอยาดี. (2547). *การศึกษาทางคลินิกเปรียบเทียบประสิทธิภาพการใช้สารเพื่อให้ผิวขาว 1 % Alpha arbutin กับ 3% Hydroquinone ในการรักษาฝ้าที่หน้า*. ปริญญานิพนธ์ วท.ม. (ตจวิทยา). กรุงเทพฯ : บัณฑิตวิทยาลัย มหาวิทยาลัยศรีนครินทรวิโรฒ. คณะกรรมการควบคุม:รองศาสตราจารย์. นายแพทย์. มนตรี อุดมเพทายกุล, รองศาสตราจารย์.นายแพทย์.ดร.ปิติ พลังวชิรา.

ฝ้าเป็นความผิดปกติของสีผิวที่พบได้บ่อย ลักษณะเป็นปื้นสีน้ำตาลอ่อนจนถึงน้ำตาลเข้มหรือเทาอมน้ำเงิน พบอุบัติการณ์ได้ในผู้ป่วยทุกเชื้อชาติและทุกเพศ พบบ่อยในเพศหญิง การรักษาฝ้าถือว่าเป็นเรื่องท้าทายของแพทย์ เพราะยังไม่มีวิธีการรักษามาตรฐานที่ได้ผลดีแน่นอน และ การเกิดซ้ำหลังการรักษาพบได้บ่อย การรักษาหลักที่นิยมในปัจจุบันคือการใช้สารกันแดดร่วมกับสารฟอกสีผิวให้ขาวขึ้น สารหลักที่ใช้คือ ไฮโดรควิโนน (hydroquinone) ความเข้มข้นร้อยละ 2-5 โดยสารนี้จะลดการทำงานของเอนไซม์ไทโรซิเนส ซึ่งมีบทบาทสำคัญต่อการสร้างเม็ดสีเมลานินที่ทำให้เกิดฝ้า เป็นสารที่ให้ผลการรักษาดีที่สุด แต่ยังมีปัญหาคือพบผลข้างเคียงได้บ่อย เช่น เกิดผื่นแพ้สัมผัส, ผื่นแพ้ระคายเคือง , ผื่นแพ้แสง เป็นต้น สารอัลฟาอาบูตินซึ่งเป็นสารเพื่อให้ผิวขาวชนิดใหม่ สกัดได้จากธรรมชาติและเป็นสารอนุพันธ์ของไฮโดรควิโนน การวิจัยนี้จึงมุ่งเน้นไปที่การประเมินประสิทธิภาพและความปลอดภัยในการรักษาฝ้าของสารอัลฟาอาบูตินเปรียบเทียบกับสารไฮโดรควิโนนและยาหลอก โดยได้ทำการทดลองทางคลินิกแบบไปข้างหน้า ในผู้ป่วยที่เป็นฝ้า 42 คน โดยแบ่งผู้ป่วยเป็น 3 กลุ่มจากการจับสลาก ผู้ป่วยแต่ละคนได้ครีมคนละ 2 หลอด โดยผู้ป่วยใช้ครีมหลอดหนึ่งทาที่หน้าซีกหนึ่งและใช้ครีมอีกหลอดทาหน้าซีกตรงข้ามวันละหนึ่งครั้งก่อนนอนเป็นระยะเวลา 10 สัปดาห์ ทำการประเมินผลโดยใช้เครื่องวัดเม็ดสี Mexameter และบันทึก MASI score ทุกสองสัปดาห์จนจบการทดลอง และประเมินผลโดยรวมอีกครั้งโดยแพทย์และผู้ป่วยหลังเสร็จสิ้นการทดลอง ผลการศึกษาพบว่าการรักษาฝ้าด้วยครีม 1% อัลฟาอาบูติน และครีม 3% ไฮโดรควิโนน ทั้งคู่มีประสิทธิภาพในการลดเม็ดสีของฝ้าได้ดีกว่าการใช้ยาหลอกอย่างมีนัยสำคัญทางสถิติ (P=0.007 และ 0.001) และเมื่อเปรียบเทียบประสิทธิภาพในการรักษาฝ้าระหว่างครีม 1% อัลฟาอาบูตินกับครีม 3%ไฮโดรควิโนนพบว่าให้ผลการรักษาที่ไม่แตกต่างกัน ส่วนผลข้างเคียงพบว่าสารไฮโดรควิโนนพบผลข้างเคียง 14.2%ในขณะที่สารอัลฟาอาบูตินพบผลข้างเคียง 4.5% ดังนั้นจากการศึกษานี้จึงสามารถสรุปได้ว่าสาร 1% อัลฟาอาบูตินมีประสิทธิภาพในการรักษาฝ้าเทียบเท่ากับสาร 3% ไฮโดรควิโนน โดยพบผลข้างเคียงที่น้อยกว่า ดังนั้นสารอัลฟาอาบูตินจึงเป็นตัวเลือกใหม่ที่น่าสนใจในการใช้รักษาฝ้า.