COMPARISON OF 10%, 20% and 40% LICORICE EXTRACT CREAM AS SKIN LIGHTENING AGENT

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ABSTRACT Introduction Ultraviolet (UV) light that penetrates the skin is absorbed by melanin to protect skin cells from the detrimental effects of UV exposure. However, in certain circumstances, abnormal pigmentation can be a severe aesthetic problem. Licorice extract as a natural source has been proven invitro to stimulate and suppress melanogenesis. **Objective** Assess the skin lightening effect of Licorice extract cream in various concentrations. **Subject and Method** Women aged 30-50 years who fulfilled the inclusion and exclusion criteria were included in the study conducted in the Department of Dermatovenerology Faculty of Medicine, Hasanuddin University from May - July 2018. Each subject received three concentrations of Licorice cream (10%, 20% and 40%) to be applied on the upper and lower arms twice a day according to the patron for four weeks. The pigmentation spot was measured using the A-One Tab Skin and Hair Diagnostic System on day 0, 14 and 28. **Results** There were in total 12 subjects aged 30-50 years. After four weeks, the three groups of concentration improved skin brightness by decreasing the spot pigmentation. The best skin brightness levels were obtained by 10%, 40% and 20% concentration, respectively. **Conclusion** Licorice extract cream with 10% concentration is more effective in lightening the skin than the concentration of 20% and 40%.

KEYWORDS Pigmentation, Licorice Extract, Liquiritin

Introduction

Prolonged exposure (photoaging) to ultraviolet (UV) light will cause oxidative stress by producing reactive oxygen species (ROS) that might trigger skin malignancy. Melanin pigmentation plays a role in absorbing UV light to protect skin cells from the adverse effects of UV exposure.[1] However, in certain circumstances, abnormal pigmentation can be a serious aesthetic problem.[2]

The use of natural materials is a way to reduce photodamage due to ROS produced induced by UV exposure. Natural ingredients usually contain flavonoids and phenolic components that have hydroxyl group aromatic rings so they can donate electrons and hydrogen to ROS, making them known as antioxidants. Glycyrrhizin is one of the main elements contained in Licorice extract. It is considered as an ingredient that can inhibit a series of lipid peroxidation reactions and inhibit free radicals. In an animal study, Glycyrrhizin proved effective as an antioxidant.[3]

Licorice extract also has other active compounds which are proven invitro to stimulate and suppress melanogenesis. Glabridin is the main component of the hydrophobic fraction of Licorice extract which works by inhibiting tyrosinase enzyme activity in B16 melanoma murine cell culture without affecting DNA synthesis. Other active compounds such as glabrene, isoliquiritigenin luciraside, isoliquiritin and licochalcone A isolated from Licorice extract also show inhibitory activity against the tyrosinase enzyme. [4] Liquiritin, one of the flavonoids contained in Licorice, does not affect tyrosinase. However, this compound causes depigmentation through other mechanisms, namely by dispersing melanin. To achieve clinical results, this active ingredient is usually applied to the skin at a dose of 1

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gr/day for four weeks. Although liquiritin extract is rather expensive, almost all cosmetic products use this ingredient in a moderate concentration. Previous studies have shown that 2% and 4% liquiritin creams applied for four weeks are effective for the treatment of melasma. [5-7]

Although studies of Licorice extract in skin lightening are found in various literature, most of the research was conducted in vitro. So far, there are only four clinical trials that have managed to show the effectiveness of Licorice in lightening the skin. The four studies used specific active compounds from Licorice extract (glabridin and liquiritin), and combined Licorice extract with other depigmented ingredients. To our knowledge, no studies have examined the skin lightening effect of Licorice extract in different concentrations and it's possible side effects when applied to skin exposed and unexposed to sunlight.

Methods

The study was conducted in the Department of Dermatovenereology, Faculty of Medicine, University of Hasanuddin and has obtained ethical approval from the Biomedical Research Ethics Commission in Hasanuddin University Medical School.

Subject

This is a clinical trial with prospective pre-and post-treatment method with a total of 12 subjects. The inclusion criteria included women aged 30-50 years with Fitzpatrick III-IV skin type (chocolate, Asian skin type), no history of cosmetic allergies, willing to use Licorice 10%, 20% and 40% cream who agreed to sign an informed consent form. Exclusion criteria include pregnant women, those with hormonal contraception, breastfeeding or undergoing hormonal therapy, having infections or other skin diseases in the target area, using skin lightening creams, and women with side effects due to the skin lightening cream being used.

All patients who met the inclusion and exclusion criteria were given 10%, 20% and 40% Licorice creams along with patrons to apply the cream in the upper arm and forearm area twice a day for four weeks. The spot pigmentation score was measured by using A-One Tab Skin and Hair Diagnostic System during the first visit, week 2, and week 4. All side effects were recorded.

Materials

Licorice extract was obtained from Xinjiang Alar Xinnong Licorice Industry Co., Ltd in powder form. The measurement of Liquiritin concentration contained in Licorice extract was done with the following stages:

1. Sample Making

   The sample in the form of Licorice extract powder weighed 40 mg in a flask measuring ten mL = 4000 ppm with replication III. For samples using 700 ppm, 0.875 ml preparation was taken and added up to 5 mL in a 5-ml-flask.

2. Comparative Standard Making

   Pure Liquiritin powder was weighed 5 mg in a flask measuring 5 mL = 1000 ppm

3. Making Standart Curve

Table 1 Dilution of Liquiritin Solution for Making Standard Curves.

Concentration (ppm)	Volume (mL) taken by pipette	End Volume (mL)
15	0.075	5
30	0.15	5
60	0.3	5
120	0.6	5
180	0.9	5

Samples and solutions of various concentrations of liquiritin were filtered using Whattman paper 0.45 nm then inserted into the HPLC vial (high-performance liquid chromatography) to measure the levels of liquiritin in the sample. The results of measurements with HPLC obtained retention time (RT) of the liquiritin compound were 22.4 minutes. Furthermore, each standard concentration of the liquiritin gives a different area to RT = 22. Minutes.

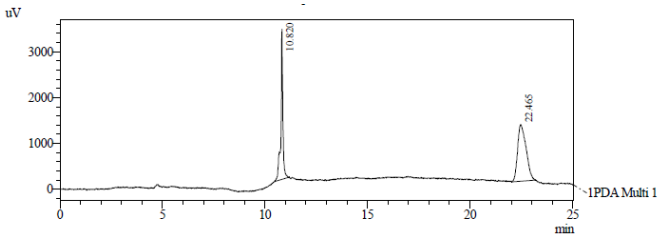

Fig.1. Pure Liquiritin Chromatogram Profile (RT 22.4 minutes) at 15 ppm Concentration Using HPLC.

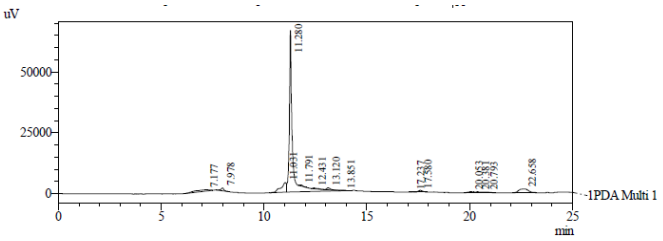

Fig.2. Chromatogram Profile of Licorice Extract Sample at 700 ppm Concentration Using HPLC.

The equation of the raw curve of the liquiritin was obtained by plotting between the concentrations of liquiritin (x) and area (y) so that the line equation can be determined y = 1536.x + 7667. Based on this equation, the concentration of liquiritin in Licorice extract was calculated at 4%.

Licorice cream was made by mixing Licorice extract powder and cream base material to obtain a concentration of 10%, 20%, and 40%. Subjects were asked to apply the three concentrations on the upper arm and forearm according to the patron.

Skin Analyzer

Brightness level was measured using the A-One Tab Skin and Hair Diagnostic System. The measured parameter is the spot pigmentation score was seen on the screen of the device.

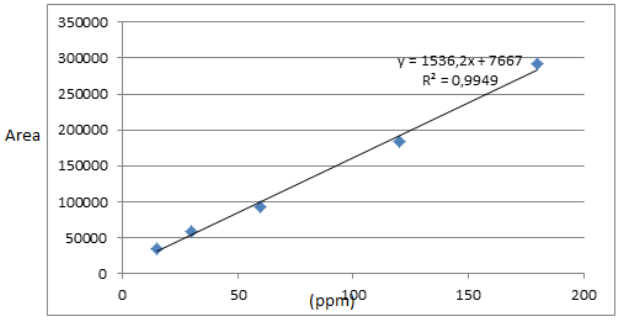

Fig.3. Liquiritin Standard Curve

Table 2 The difference in spot pigmentation scores between the three groups of Licorice cream concentrations before treatment (Day 0).

Concentration	Spot pigmentation score day -0		p*
	Min-Max	Median	
10%	1-14	6.5	
20%	2-17	5.5	0.73
40%	1-17	6.0	
* Kruskal-Wallis test; The p column superscript shows that the Kruskal Wallis test results are not significantly different (p>0.05).			

Results

Form a total of 12 research subjects; it was found that there were two people in the 30-35 years age group (16.7%), nine people in the 35-40 years age group (75%), and one person in the 41-45 years age group (8.3%). Eight people were students (66.7%) while the remaining four people were janitors (33.3%).

The results of the analysis in table 2 show that the spot pigmentation score before the application was not significantly different in the three groups of concentrations of Licorice cream. This shows that the initial score of spot pigmentation scores from the three groups of cream concentrations did not influence the differences in the effect of the concentration of the Licorice cream on changes in spot pigmentation scores after administration of Licorice.

From the summary of the results analysis in table 3, it is shown that there was a change in the spot pigmentation scores (increase in skin brightness) in the three concentration groups on the 14th day of administration of the cream; 2.0 units each for 10% cream; 1.5 units for 20% cream and 1.0 unit for 40% cream. However, only the 20% concentration cream showed a statistically significant effect. The results of the Kruskall Wallis test which analyzed the differences in the decrease in spot pigmentation scores (increase in skin brightness) between the three concentrations showed no significant difference (p> 0.05) after 14 days of applying the cream.

Table 4 shows a significant decrease in the spot value of pigmentation (increase in brightness) (p <0.05) from each concen-

Table 3 Comparison of Changes in spot values of pigmentation Day 0 and 14 to LICORICE extract cream 10%, 20% and 40%

Concentration	Median (Min-Max)		Changes median (d)	p*
	H0	H14		
10%	6,5 (1-14)	4,5 (1-12)	2^a	0.097
20%	5,5 (2-17)	4,0 (1-17)	$1,5^a$	0.035
40%	6,0 (1-17)	5,0 (1-17)	$1,0^a$	0.129
*Wilcoxon test; The same superscript in the change column shows that the Kruskal Wallis test results were not significantly different (p> 0.05).				

Table 4 Comparison of Changes in spot pigmentation values Day 14 and 28 for LICORICE extract cream 10%, 20% and 40%

Concentration	Median (Min-Max) Spot Pigmentation		Score Changes in Spot Pigmentation (d)	p*
	H14	H28		
10%	4,5 (1-12)	3 (1-8)	$1,5^a$	0.003
20%	4,0 (1-17)	3 (1-9)	$1,0^a$	0.020
40%	5,0 (1-17)	3 (1-11)	$2,0^a$	0.011
* Wilcoxon test; The same superscript in the score changes column shows that the Kruskal Wallis test results were not significantly different (p> 0.05).				

tration from day 14 to day 28 of applying cream; 1.5 units each for cream 10%; 1.0 unit for 20% cream; 2.0 units for 40% cream. However, the results of the Kruskal Wallis test at the three cream concentrations did not show a significant difference (p> 0.05) from the 14th day to the 28th day. This suggests that the effect of Licorice cream on decreasing the spot pigmentation score (increase in skin brightness) is not affected by the concentration of Licorice cream from the 14th day to the 28th day.

Table 5 Comparison of Changes in Spot Pigmentation Value Day 0 and 28 to Licorice Extract Cream 10%, 20% and 40%

Concentration	Median (Min-Max) Spot Pigmentation Score		Score Changes in Spot Pigmentation (d)	p*
	H0	H28		
10%	6,5 (1-14)	3 (1-8)	3,5[a]	0,001
20%	5,5 (2-17)	3 (1-9)	2,5[a]	0,000
40%	6,0 (1-17)	3 (1-11)	3,0[a]	0,001
*Wilcoxon test; The same superscript in the change column shows that the Kruskal Wallis test results were not significantly different (p> 0.05).				

Table 5 shows a significant decrease in the spot pigmentation score (increase in brightness) (p <0.05) from each concentration; from day 0 to day 28; 3.5 units for cream 10%; 2.5 units for 20% cream; 3.0 units for 40% cream. However, the results of the Kruskal Wallis analysis on the three cream concentrations did not show a significant difference (p> 0.05) from day 0 to day 28. This implies that the effect of Licorice cream on decreasing the spot value of pigmentation (increase in skin brightness) is not affected by the concentration of Licorice cream from day 0 to day 28.

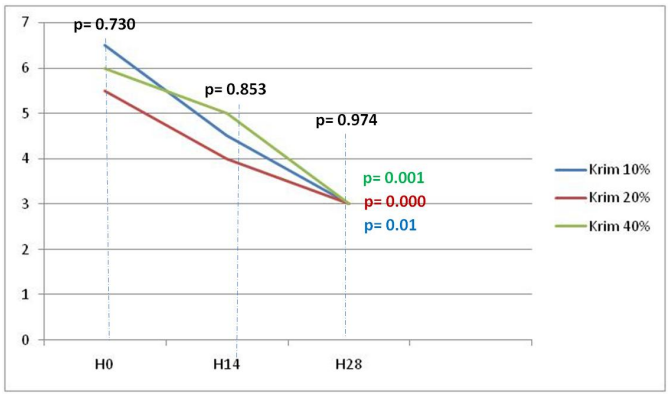

Fig.4. Comparison of Changes in Spot Pigmentation Score Day 0 and 28 to Licorice Extract Cream 10%, 20% and 40%

Discussion

This study involved 12 women with dull skin colour according to the Glogau criteria. The youngest sample was 34 years old, and the oldest was 45 years old. This is by the literature which states that extrinsic ageing may have begun in the second decade with symptoms of skin discolouration in the form of hyperpigmented patches, wrinkles, dry skin and skin tumours. In a study conducted by Goh (1990) in Asian populations (China, Malaysia, Indonesia), it was found that earliest characteristic of early ageing was hyperpigmentation, [8] with a higher frequency found in women compared to in men. [9] One of the factors causing premature ageing is hormonal factors in women such as estrogen, progesterone, testosterone, DHEA, premenopause and menopause. In women who have entered the menopausal period, there is a decrease in ovarian function resulting in less estrogen production. As a result, the skin becomes smooth and look dull. [10]

In this study, applying Licorice extract cream of different concentrations twice a day for 28 days showed a significant decrease in pigmentation score (p <0.05). From the data in table 6, it was evident that 20% Licorice extract cream has the smallest p-value, suggesting that it might possess the best effect among the three concentrations. This result is by the study conducted by Amer and Metwali (2000) who used a 20% Licorice extract cream in 20 women with melasma for four weeks. Although Licorice 20% extract cream was seen to have the smallest p-value, the effectiveness was not significantly different compared to 10% and 40% concentrations. This is by the literature which states that Licorice extract in the concentration range of 10-40% is effective in inducing skin lightening. [11]

Licorice extract has been known as the safest skin lightening material and has the least side effects. [6] This material works by inhibiting the tyrosinase enzyme and several other enzymes in the arachidonic acid cascade, especially the cyclooxygenase enzyme released after exposure to sunlight, making it also considered to possess an anti-inflammatory effect. Therefore, Licorice is also effective to treat hyperpigmentation due to sun exposure. Also, another component that has a depigmentation effect is liquiritin which works by dispersing melanin.[12, 13] Some chemical constituents of Licorice such as polyphenolic flavonoids are known as antioxidant agents. [14]

Liquiritin has been used in several clinical trials to assess the efficacy of skin brightness. Licorice extract used in research by Amer and Metwali had a 2% liquiritin level and was proven to brighten the skin in four weeks. A similar result was shown by Zubair and Mujtaba who compared the efficacy of 2% and 4% liquiritin to 4% hydroquinone, which was the gold standard for the melasma treatment in Pakistan. After eight weeks of treatment, more uniform skin brightness and the color was obtained after 4% liquiritin application compared to 4% hydroquinone. [15]

A study conducted by Akram et al. comparing the efficacy of 4% liquiritin with a mixture of 4% liquiritin and 5% ascorbic acid showed a similar result, showing that the combination of liquiritin and ascorbic acid was more effective after eight weeks of application. [7]

The level of liquiritin from Licorice extract in this study was 4% (4 g of liquiritin in 100 gr of dried Licorice extract). The liquiritin level was not calculated in each cream concentration group because the compound is bound to the cream base and might decompose due to heating and acidification. Thus, the levels of liquiritin in this study may vary; higher or lower than the empirical level. [16] Low levels of liquiritin in Licorice extract

used in this study did not reduce the efficacy of lightening the skin. This is understandable because Licorice has several active compounds which also have the ability to inhibit melanogenesis.

The main active compound in Licorice is glabridin which has been shown to inhibit tyrosinase T1 and T3 isoenzymes in melanoma B16 cells without affecting DNA synthesis. Yokota et al. showed that UV-induced pigmentation and erythema can be inhibited by applying glabridin 0.5%. [17] The depigmentation effect of glabridin is known to be 16-fold stronger than hydroquinone. Also, glabridin has a more rapid onset of action. In one study, pure glabridin appeared to have the effect of brightening the skin after seven days of applying. [11, 15]

Other compounds that can inhibit the tyrosinase enzyme are glabrene and isoliquiritigenin, which inhibitory activity depends on the dose used. [4]

Glycyrrhizinate acid can potentiate the work of hydrocortisone by inhibiting 11β-hydroxysteroid dehydrogenase (11β-OHSD), hence reducing the conversion of cortisol to cortisone.[18] In the form of stearyl, glycyrrhetinic acid is commonly used as a sun care agent and sunscreen. [19]

Licochalcone A (LicA) contained in Licorice has been known to have anti-inflammatory and anti-oxidative properties that can increase the ability of skin cells to withstand the harmful effects of UV light. The anti-inflammatory and anti-oxidative properties of LicA have been associated with inhibitory activity in NFkB signalling.

The limitations of this study are the odour, colour and consistency of the cream. The appearance of the cream should be improved so that the physical properties of the cream do not interfere with daily activities.

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