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FORMULATION OF HYPOPIGMENTATION CREAM AND EVALUATION OF ITS EFFECT ON SKIN PIGMENT. PART I: FORMULATION OF THE PRODUCT

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Abstract

Introduction: Melasma is a commonly acquired hypermelanosis of facial skin due to various etiological factors including hormonal imbalance. Although it affects any one is particularly common in women, especially pregnant women and those who taking oral or patch contraceptives or hormone replacement therapy.

Aim: This research aimed to formulate stable water in oil (w/o) cream containing plant extract of Glycyrrhiza glabra as active material obtained by concentrating the alcoholic extract of the plant roots, was entrapped in the inner aqueous phase of w/o cream.

Material and Methods: Base containing no active material and a formulation containing ethanolic extract of the plant which was prepared in Samarra Drugs Industry laboratories. Samples of base and formulation were stored at different accelerated conditions (8°C, 25°C, 30°C, 40°C, 40°C +75% RH) for four weeks to predict the stability of the creams.

Results: It was concluded that the formulation was stable chemically and physically over the studied storage conditions and without induction of allergic or contact dermatitis.

Key words: Melasma; plant extract; topical treatment

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Introduction

The term melasma is derived from the Greek word 'melas' meaning black [1]. It is a disorder of pigmentary system characterized by irregular brown or greyish-brown, acquired hypermelanosis of sun-exposed areas especially the face [2]. Melanin largely decides our skin color and is an essential defense mechanism against the sun for us. Melanin also overproduces with acne and this is what causes the dark skin spots to remain long after the red acne spots have gone [1]. Topical polyherbal formulations are the latest additions to the dermatologists repertoire of treatment. Scientists have known for decades that some plants are amazingly good at reducing and diverting the production of Melanin [3]. Particularly advantageous cosmetic emulsion preparations are obtained when antioxidants are used as active ingredients [4]. Many antioxidatively acting compounds are isolated from natural herbs and spices (extracts) and used as potential antioxidants in cosmetics [5]. An extract of *Glycyrrhiza glabra* is rich of natural antioxidants [6].The best natural antioxidants in extract of *Glycyrrhiza glabra* are glycyrrhizin (glycyrrhizic acid) and flavonoids [7]. The role of plant extract on skin is mainly attributed to its antioxidant activity particularly to its potent antioxidants triterpene, saponins and flavonoids [8]. Skin whitening [9], skin depigmenting [10], skin lightening [11], antiaging [12], anti-erythemic [13], emollient [14], anti-acne [15] and photoprotection effects [16,17].

Materials and Methods

Materials

The materials used in this study were supplied by the State Company for Drugs Industries (SDI) which include the following:

· Paraffin oil and coconut oil. (Wacker Chemicals Ltd./Germany)
· Cetomacrogol 1000. (Merck KGa/Germany)
· Cetostearyl alcohol. (Merck KGa/Germany)

· Beeswax. (Merck KGa/Germany)
· Glycerin. (Merck KGa/Germany)
· Lemon oil. (Merck KGa/Germany)
· Distilled Water.
· Extract of Glycyrrhiza glabra (ethanolic) is to be prepared by in the State Company for Drug Industries.

Preparation of Base and Formulation
Water in oil (W/O) cream was prepared by the addition of aqueous phase to the oily phase with continuous agitation. To prepare base(placebo); oily phase that consisted of paraffin oil, beeswax, coconut oil and surfactants (cetomacrogol 1000 and cetosteatyl alcohol), is heated up to 75°C±1°C. Aqueous phase consisting of glycerin and water is heated to the same temperature. The formulation was also prepared by same method; the only difference is the addition of *Glycyrrhiza glabra* extract (active drug) that is added in aqueous phase consisting of glycerin and water. Each formulation consists of preserved water (propyl paraben 0.02% w/w and methyl paraben 0.1% w/w) to 100g. The formulations were neutralized by Triethanolamine to pH=5.5 at 25°C (Tabl. I).

Patch Test
On the first day of skin testing, patch tests are to be performed on the forearms of each volunteer. 5cm x 4cm regions were marked on both the forearms. Basic values for erythema and melanin are to be measured with the help of Mexameter. 1.0g of base and formulation each are applied to the 5cm X 4cm marked regions separately on each forearm. The regions are covered with the surgical dressing after application. After 24 hours, dressings are removed and the measurements of erythema and melanin are repeated on both forearms.

Panel Test
Every individual is provided with a form prepared previously to test the sensory values of cream. This form consisted of parameters to be evaluated and every parameter is assigned 11 values from –5 to +5 indicating very bad to very good, respectively. This form is asked to be completed independently by each individual on day 28.

Dermatological tests
Erythema of the skin are determined on the first day before application of any cream and then on days 7, 14, 21 and 28.

Parameters for Evaluation of Formulation Characteristics These parameters include

Centrifugation Tests for Creams:
Centrifugation test is to be performed for both the base and formulation kept at different storage conditions up to a period of 28 days at different time intervals. Phase separation on centrifugation is to be recorded in any of the samples kept at different storage conditions i.e. 8^0C, 25^0C, 40^0C and 40^0C+ 75% relative humidity up to 28th day of observation. This parameter indicate both creams stability at all the storage conditions for 28 days. It is evident that proper homogenization speed during emulsion formulation prevented the base and formulation breakage during stress conditions.

Stability Tests
Physical analysis, types of cream, pH determination, was analyzed to assure the formulation of desired properties. Stability tests were performed at different conditions for cream to note the effect of these conditions on the storage of creams. These tests on samples were kept at 8°C ± 0.1°C (in refrigerator), 25°C ± 0.1°C (R.T), 30°C(in oven), 40°C ± 0.1°C (in oven) and 40°C ± 0.1°C (in oven) with 75% relative humidity (RH). Samples are observed with respect to change in color, liquefaction and phase separation.

Color
The freshly prepared base is creamy white while formulation is pale yellow in color (due to the presence of *Glycyrrhiza glabra* extract). It is presumed that there is no change in color of any sample of base and formulation at different storage condition i.e. 8^0C, 25^0C, 40^0C and at 40^0C+ 75% relative humidity up to the observation period of 28 days.

Liquefaction
Liquefaction is to be observed in any of the sample of base and formulation kept at 8^0C and 25^0C during whole observation period of 28 days.

Phase Separation
Phase separation was observed in any of samples of base and formulation kept at 8^0C, 25^0C, 40^0C and at 40^0C + 75% relative humidity up to observation period of 28 days.

Formula	Composition % (W/W)						
	Licorice	Glycerin	Paraffin oil	Coconut oil	Beeswax	Cetoma-crogol 1000	Cetosteatyl Alcohol
F 1	1.0	18	20	3.0	5.0	1.0	5.0
F2	1.0	18	20	3.0	5.0	1.5	5.0
F3	1.5	18	20	2.0	5.0	1.8	6.0
F4	1.5	18	20	1.0	5.0	1.8	6.0
F5	2.0	18	20	1.0	5.0	2.0	6.0
F6	2.5	18	20	1.0	5.0	2.0	6.0
placebo	0.0	18	20	1.0	5.0	2.0	6.0

Table I. Formulations composition.

pH
Base and formulation pH was measured during the storage period and at different storage temperatures.

Erythema
The side effect of base and formulation as presented by contact or allergic dermatitis was tested. The indicator used to determine such side effect was the recording of erythema following application of base and formulation to the skin of the volunteers.

Ethical Approval
The research protocol was approved by the ethical committee of Tikrit University College of Medicine and informed consent was taken from any individual shared in the study.

Results

Physical analysis

Color
The freshly prepared base is creamy white while formulation is pale yellow in color (due to the presence of *Glycyrrhiza glabra* extract). There is no change in color of any sample of base and formulation at different storage condition i.e. 8°C, 25°C, 30°C, 40 °C and at 40°C+ 75% relative humidity up to the observation period of 28 days.

Liquefaction
No liquefaction was observed in any of the sample of base and formulation kept at 8°C and 25°C and 30°C during whole observation period of 28 days but slight liquefaction was observed in samples kept at 40°C and 40°C + 75% RH from 21st day of observation but there was no increase in liquefaction till the end of study period.

pH Measurements
The pH is a significant parameter in so far as the effectiveness of the cream is concerned and it can be used as an indicator of the formulation stability. All the samples have a pH close to the skin pH with range 5.5 for the formulation and decreased in case of base to 4.9 during the observation of study period as shown in Table II and III.

Phase Separation
Phase separation was not observed in any of samples of base and formulation kept at 8°C, 25°C and 30°C, but there was a little bit of separation in samples kept at 40°C and 40°C + 75% RH from the 21 day of observation.

Erythema
For confirming the safety of dermatological preparations, the important point is that must not cause any contact dermatitis when applied to the skin. In this study it was found that erythema contents were decreased from 1st to 4th week after the application of base and formulation.

Patch Test
On the first day of skin testing, patch tests was performed on the forearms of each volunteer. 5cm x 4cm regions were marked on both the forearms. Basic values for erythema are to be measured with a ruler. 1.0g of base and formulation each were applied to the 5cm x 4cm marked regions separately on each forearm. The regions were covered with the surgical dressing after application. After 24 hours, dressings were removed and the measurements of erythema were repeated on both forearms.

Time	8°C	25°C	30°C	40°C	40°C+ RH	Mean
	For formulation					
0.00	5.44	5.38	5.41	5.6	5.82	5.53
1st week	5.53	5.61	5.45	5.37	5.46	5.48
2nd week	5.4	5.58	5.7	5.58	5.72	5.67
3rd week	5.41	5.6	5.63	5.49	5.52	5.53
4th week	5.32	5.35	5.44	5.63	5.52	5.5
Mean	5.5	5.49	5.52	5.53	5.6	5.54
For base						
0.00	5.76	5.63	5.66	5.51	5.53	5.65
1st week	5.43	5.37	5.25	5.2	5.17	5.2
2nd week	5.2	5.12	5.07	5.1	5.0	5
3rd week	4.9	4.76	4.73	4.59	4.51	4.6
4th week	4.53	4.41	4.37	4.25	4.08	4.3
Mean	5.1	5	4.97	4.91	4.82	4.9

Table II. Measurements pH values of formulation and base during 28 days at different study conditions.

Time	8°C	25°C	30°C	40°C	40°C+ RH	Mean	*p*
	For formulation						
0.00	5.44	5.38	5.41	5.6	5.82	5.53	NS
1st week	5.53	5.61	5.45	5.37	5.46	5.48	NS
2nd week	5.4	5.58	5.7	5.58	5.72	5.67	NS
3rd week	5.41	5.6	5.63	5.49	5.52	5.53	NS
4th week	5.32	5.35	5.44	5.63	5.52	5.5	NS
Mean	5.5	5.49	5.52	5.53	5.6	5.54	
P	NS	NS	NS	NS	NS		
For base							
0.00	5.76	5.63	5.66	5.51	5.53	5.65	NS
1st week	5.43	5.37	5.25	5.2	5.17	5.2	NS
2nd week	5.2	5.12	5.07	5.1	5.0	5	NS
3rd week	4.9	4.76	4.73	4.59	4.51	4.6	NS
4th week	4.53	4.41	4.37	4.25	4.08	4.3	NS
Mean	5.1	5	4.97	4.91	4.82	4.9	
P	NS	NS	NS	NS	NS		

Table III. Measurements pH values and p values of formulation and base during 28 days at different study conditions.

Discussion

Every case of melasma starts off in the epidermis, where melanocytes are actively producing pigment [18]. A normal case of melasma can turn into dermal melasma if skin becomes over- irritated and inflamed. When this happens, it causes a temporary split between the dermis and epidermis. During this time, hyperpigmented cells can drop from the epidermis into dermis [19]. Once in the dermis, these cells become very resistant to topical treatment. Dermal melasma is particularly difficult to treat since active tyrosinase activity is only found in epidermal melanocytes. In dermal melanin, tyrosinase activity is not present; therefore dermal melasma is resistant to topical treatment [20].

The current work aimed to formulate a stable water in containing *Glycyrrhiza glabra* (*G. glabra*) extract and studying its effect on skin pigment (Melanin). Base containing no active material and formulation containing alcoholic extract of *G. glabra* at different concentrations. Six formulations were prepared and formulation number (6) was selected depending on a pilot study which included 50 volunteers who were intended to the daily dermatology clinics.

No phase separation was seen in any of samples of base and formulation kept at 8°C, 25°C and 30°C, but there was a little bit of separation in samples kept at 40°C and 40°C +75% RH from the 21 day of observation. This indicated both creams were stable at 8°C, 25°C and 30°C. Proper homogenization speed during cream formulation prevented the base and formulation breakage during stress conditions [21].

In the present both base and formulation did not show any change in color of samples at different storage conditions up to the observation period of 28 days. *G. glabra* extract containing poly phenols that have antimicrobial activities [22] and thus prevent color change of the formulation. In addition, no liquefication was observed in any of the sample of the base and formulation kept at 8°C, 25°C and 30°C. However, slight liquefication was observed in samples kept at 40°C and 40°C+RH after 3 weeks of observation, but there was no increase in liquefication till the end of study period. Thus, the above findings (color, liquefication and phase separation) indicated that both base and formulation were stable at 8°C, 25°C and 30°C storage conditions for 28 days.

pH is a significant parameter of effectiveness of cream stability [23] and it was a key indicator factor of aqueous phase [24]. Skin pH range between 5 and 6, and 5.5 is considered to the average pH of the skin [25]. In the present study, the pH of the freshly prepared base and formulation was 5.65 and 5.53, this is very close to the skin pH. The pH of the base kept at different storage conditions was found to be decreased gradually over storage period. However, the change in pH was not statistically significant over time. Different storage temperatures show no significant changes in pH. The formulation samples pH was with a non- constant pattern (i.e. increased or decreased), however, there was a non significant pH changes over time and also insignificant at different storage temperature. The change in pH of formulation may be due to presence of *G. glabra* extract [24].

In the present study indicated that erythema induced by application of base and formulation was decreased after 24 hr. of their application. Thus it is concluded that both base and formulation produced non skin irritation after performing patch test of 24 hrs. It may be attributed to the presence of a good emollient glycerin in the base and formulation, and/or *G. glabra* [26] in the formulation, which has the ability to reduce skin erythema [27].

An important criterion for any cosmetic is that it must not cause any contact dermatitis following application to the skin. Contact dermatitis was not always due to ingredients of the cosmetic.

Environmental factors, skin types, cosmetic misuses and physical conditions may all causes contact dermatitis [28]. Skin irritation is caused by a direct toxicity of chemicals on cells or blood vessels in the skin and is different from contact allergy which is caused by immune response [29].

The present study was indicated that erythema induced by application of base and formulation decreased over time. The decrease in erythema over time may be due to in the presence of coconut oil in base which is a good emollient [30] and decrease inflammation [31]. In addition, presence of *G. glabra* extract in the formulation may act as soothe and calm agent to the skin [32].

In conclusion, the hypopigmentation formulation presented in this study was stable physically and chemically and not induced contact or allergic dermatitis and may be suitable as treatment for melasma.

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