2011 International Conference on Bioscience, Biochemistry and Bioinformatics
IPCBEE vol.5 (2011) © (2011) IACSIT Press, Singapore

GLYCYRRHIZA GLABRA EXTRACT CREAM: EFFECTS ON SKIN PIGMENT "MELANIN"

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***Abstract*—The current work aimed to formulate a stable w/o cream containing Glycyrrhiza glabra extract and studying its effects on skin pigment "melanin". Glycyrrhiza glabra Extract obtained by concentrating the alcoholic extract of Glycyrrhiza glabra roots, was entrapped in the inner aqueous phase of W/O emulsion. Base containing no active material and a formulation containing ethanolic extarct of Glycyrrhiza glabra (1%) were prepared. Samples of base and formulation were stored at different accelerated conditions (8°C, 25°C, 40°C, 40°C+75%RH) for four weeks to predict the stability of creams. Base and formulation were stable at all accelerated conditions regarding color, liquifaction and phase separation. Both base and formulation were applied to the cheeks of human volunteers for four weeks. Different parameters of human skin like melanin, erythema were monitored every week to measure any effect produced by these creams. Significant decrease in skin melanin was produced by formulation, where as insignificant decrease in this skin pigment was observed by base. Both creams were good for sensory evaluation.**
Keywords- Glycyrrhiza glabra extract, W/O cream, Skin melanin, Erythema, Skin moisture, Skin sebum, Skin pH.

I. INTRODUCTION

Emulsions usually consist of mixtures of an aqueous phase with various oils and/or waxes. The liquid that is broken up into droplets is termed the internal or dispersed phase, whereas the liquid surrounding the droplets is known as the external or continuous phase, both phases are held together by a third component the surface active agent also referred to as emulsifier, which is necessary to stabilize the emulsion [1].

Most of products which are used in cosmetics are emulsions [2]. The basic components of these formulations are emulsifiers, humectants and viscosity modifiers. Additional value can be given to these formulations by including active ingredients with specific cosmetic effects [2]. Particularly advantageous cosmetic emulsion preparations are obtained when antioxidants are used as active ingredients [3]. There is a growing interest in natural antioxidants found in plants. Many antioxidatively acting compounds are isolated from natural herbs and spices (extracts) and used as potential antioxidants in cosmetics [4].

An extract of Glycyrrhiza glabra is rich of natural antioxidants. The best natural antioxidants in extract of Glycyrrhiza glabra are glycyrrhizin (glycyrrhizic acid) and flavonoids [5]. Glycyrrhiza glabra extract is obtained from the roots of Glycyrrhiza glabra by solvent extraction [6] method and then concentrating the extract [7] by rotary evaporator. Glycyrrhiza glabra extract is preserved by refrigeration and/or freezing. The role of *Glycyrrhiza glabra* extract on skin is mainly attributed to its antioxidant activity particularly to its potent antioxidants triterpene saponins and flavonoids [5]. Skin whitening [8], skin depigmenting [9], skin lightening [10, 11], antiaging, anti-erythemic [12], emollient [13], anti-acne [14] – [15] and photoprotection effects are mainly attributed to *Glycyrrhiza glabra* extract.

Glycyrrhiza glabra extract is incorporated in internal aqueous phase of W/O emulsion. The aim of this study was to formulate a stable W/O cream and to measure the effects of this W/O cream of *Glycyrrhiza glabra* extract on different physiologic functions of skin particularly skin melanin as well as erythema, skin moisture, skin sebum, pH of human skin and Transepidermal water loss (TEWL).

II. MATERIALS AND METHODS

A. Materials

Paraffin oil and coconut oil were obtained from Merck (Germany). Abil-EM 90 was purchased from Franken Chemical (Germany), Beeswax from Fluka (Switzerland), Glycerin from Lever Brothers (Pakistan), Lemon oil (Pakistan) while Distilled Water was prepared in the labs of Pharmacy department, the Islamia University of Bahawalpur, Pakistan. Extract of Glycyrrhiza glabra (ethanolic) was prepared in laboratory of Pharmacy department, the Islamia University of Bahawalpur, Pakistan.

B. Methods

1) Preparation of Extract of Glycyrrhiza glabra

Glycyrrhiza glabra roots were used as plant material. The identification of this plant was performed by Cholistan Institute of Desert Studies at The Islamia University of Bahawalpur, Pakistan.

The Shade dried roots of *Glycyrrhiza glabra* (Family: Fabaceae) were taken, cleaned and ground in an electric grinder to have coarse powder. The powder was then passed through sieve number 60 and stored in a well-closed

container at 25°C. One kg powdered material of *Glycyrrhiza glabra* roots was put into a glass flask and analytical grade ethanol was added, soaked for three days and kept in the laboratory. The flask was shaken for 10 minutes after each day for three days. Finally the soaked material of plant was filtered through several layers of muslin cloth one by one for coarse filtration. The filtrate so obtained was evaporated under reduced pressure at 45 ºC in a Rotary vacuum evaporator (Eyela, Japan). The process of evaporation was continued till little amount of alcohol was remained. Then the same procedure of soaking, filtering and evaporating was repeated with the remaining material in the flask for two times more. Then, the final concentrate of three successive extractions was combined and again concentrated on Rotary vacuum evaporator under reduced pressure at 45ºC. The syrupy extract so obtained was collected in glass containers and stored in freezer at 0^{o}C.

2) Preparation of Base and Formulation

In this study, W/O creams were prepared by the addition of aqueous phase to the oily phase with continuous agitation [16]. To prepare base; oily phase that consisted of paraffin oil, beeswax, coconut oil and surfactant (ABIL-EM 90), was heated up to $75^{0}C \pm 1^{0}C$. At the same time, aqueous phase consisting of glycerin and water was heated to the same temperature. After that, aqueous phase was added to the oil phase drop by drop. Stirring was continued at 2000 rpm by the mechanical mixer for about 10 minutes until complete aqueous phase was added. After the complete addition of the aqueous phase, the speed of the mixer was reduced to 1000 rpm for homogenization. Then triethanolamine was added and homogenization was carried out for a period of 5 minutes, then the speed of the mixer was further reduced to 500 rpm for 5 minutes for complete homogenization; until the formed cream cooled to room temperature [16].

The formulation was also prepared by same method; the only difference was the addition of *Glycyrrhiza glabra* extract (active drug) that was added in aqueous phase consisting of glycerin and water [16].

3) Stability Tests

Physical analysis, types of emulsion, pH determination, electrical conductivity and centrifugation tests of creams were analyzed to assure the formulation of desired properties. Stability tests were performed at different conditions for emulsions to note the effect of these conditions on the storage of creams. These tests were performed on samples kept at $8^{0}C \pm 0.1^{0}C$ (in refrigerator), $25^{0}C \pm 0.1^{0}C$ (in oven), $40^{0}C \pm 0.1^{0}C$ (in oven) and $40^{0}C \pm 0.1^{0}C$ (in oven) with 75% relative humidity (RH). Physical characteristic of creams, i.e. color, creaming and liquefaction were noted at various intervals for 28 days [16].

4) Product Evaluation on Skin

9 male volunteers were selected whose ages were in between 20 and 25 years. Prior to the tests, the volunteers were examined by a cosmetic expert for any serious skin disease or damage especially on cheeks and forearms. Before the study, every volunteer was provided with a volunteer protocol. This protocol stating the terms and conditions of the testing were signed by every volunteer individually. Volunteers were not informed about the contents of formulations. All the skin tests were done at 250C and 40% relative humidity conditions. On the first day, patch test (Burchard test) was performed on the forearms of each volunteer to determine any possible reactions to the emulsions. On the second day, each volunteer was provided with two creams. One cream was base and the other one was formulation containing the active ingredients. Each cream was marked with "right" or "left" indicating application of that cream to the respective cheek. The creams were applied by the volunteers themselves as instructed for 28 days. Every individual was instructed to come on days 7, 14, 21 and 28 for the skin measurements.

5) Patch Test (Burchard Test)

On the first day of skin testing, patch tests were performed on the forearms of each volunteer. 5cm X 4cm regions were marked on both the forearms. Basic values for erythema and melanin were measured with the help of Mexameter. 1.0 g 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 and melanin were repeated on both forearms.

6) Panel Test

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

7) Dermatological tests

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

8) Statistical Analysis

The measured values obtained for different parameters (melanin, erythema) were analyzed using SPSS 12.0 on the PC computer (paired samples t-test for variation between the two preparations; two-way ANOVA for variation between different time intervals).

III. Results And Discussion

A. Centrifugation Tests for Creams

In this study centrifugation test was performed for both the base and formulation kept at different storage conditions up to a period of 28 days at different time intervals.

No phase separation on centrifugation was seen in any of the samples kept at different storage conditions i.e. $8^{0}C$, $25^{0}C$, $40^{0}C$ and $40^{0}C$+ 75% relative humidity up to 28th day of observation .This indicated both creams were stable 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 [17].

B. Electrical Conductivity Tests

In this study conductivity test was performed for both base and formulation kept at different storage conditions up to a period of 28 days at different time intervals. No electrical conductivity was seen in any of the samples of base and formulation 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 indicated both the creams were stable at all the storage conditions for 28 days.

C. Stability

In this work, both base and formulation were divided in to four samples separately and these samples were kept at different storage conditions i.e. at 8^0C in refrigerator, at 25^0C, 40^0C and at 40^0C + 75% relative humidity (RH) in stability chambers. These samples at different storage conditions were observed for a period of 28 days at different intervals. Samples were observed with respect to change in color, liquefaction and phase separation [16].

D. Color

The freshly prepared base was creamy white while formulation was pale yellow in color (due to the presence of Glycyrrhiza glabra extract). There was no change in color of any sample of base and formulation at different storage condition i.e. 8^0C, 25^0C, 40 ^{0}C and at 40^0C+ 75% relative humidity up to the observation period of 28 days. Polyphenols which are present extract of Glycyrrhiza glabra have microbicide activities against huge no of bacteria [18].

E. Liquefaction

No liquefaction was observed in any of the sample of base and formulation kept at 8^0C and 25^0C during whole observation period of 28 days but slight liquefaction was observed in samples kept at 40^0C and 40^0C + 75% RH from 21st day of observation but there was no increase in liquefaction till the end of study period.

F. Phase Separation

No phase separation was observed in any of samples of base and formulation kept at 8^0C, 25^0C, 40^0C and 40^0C+ 75% relative humidity up to observation period of 28 days. This indicated both base and formulation were stable at all the storage conditions for 28 days.

G. pH

The pH is a significant parameter insofar as the effectiveness of the cream is concerned and it can be used as an indicator of emulsion stability [19]. For the formation of stable emulsions pH value of aqueous phase is the key factor [20]. pH of skin ranges between 5 and 6, and 5.5 is considered to be average pH of the skin[16]. Therefore, the formulations intended for application to skin should have pH closer to this range.

In this study, the pH of freshly prepared base and formulation was 5.51 and 5.34 respectively, which is very close to the skin pH. The pH values of the samples of base kept at different storage conditions i.e. 8^0C, 25^0C, 40^0C and 40^0C + 75% RH was found to be decreased gradually from the first day to the last day & on 28th day pH of base was 5.33, 5.31, 5.33 and 5.24 respectively**,** whereas pH of the samples of formulation kept at different storage conditions i.e. 8^0C, 25^0C, 40^0C and 40^0C + 75% RH was 4.40, 4.80, 4.50 and 4.99 respectively as represented in fig.**1,2,3,&4.**

By using two-way analysis of variance (ANOVA) technique at 5% level of significance, it was found that the change in pH of different samples of base was insignificant at different levels of time and significant at different levels of temperature but there was significant difference in change of pH of different samples of formulation at different levels of time and insignificant at different levels of temperature. When LSD test was applied to check the individual average effects of the pH of the samples of base at different temperatures with the passage of time by taking average pH values of Zero Hour at different temperatures as standard, it gives significant change at 4th week of study period at temperature level. From LSD it is concluded, that there was insignificant difference in pH values of sample of base at different time intervals but a significant difference was observed in case of different samples of formulation at different time intervals at different storage conditions with the passage of time. The decrease in pH of formulation with the passage of time may be due to the presence of Glycyrhiza glabra extract [21].

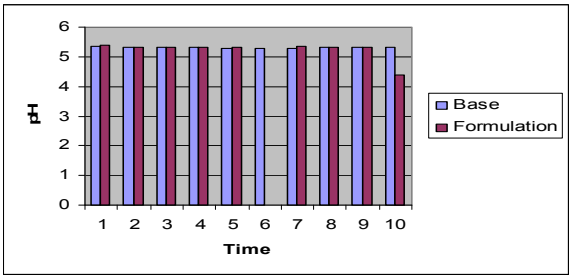

Figure 1. pH Values of Base and Formulation Kept at 8°C. Here 1=0 Hour, 2 =12 Hour, 3=24 Hour, 4=36 Hour, 5=48 Hour, 6=72 Hour, 7=7 Days, 8=14 Days, 9=21 Days, 10=28 Days.

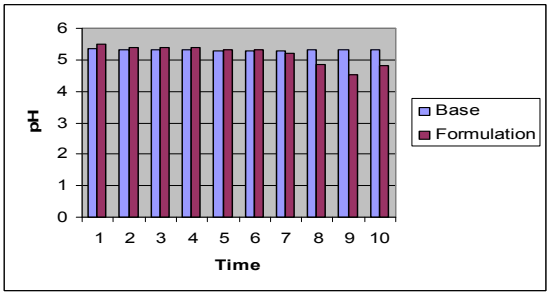

Figure 2. pH Values of Base and Formulation Kept at 25°C. Here 1=0 Hour, 2 =12 Hour, 3=24 Hour, 4=36 Hour, 5=48 Hour, 6=72 Hour, 7=7 Days, 8=14 Days, 9=21 Days, 10=28 Days.

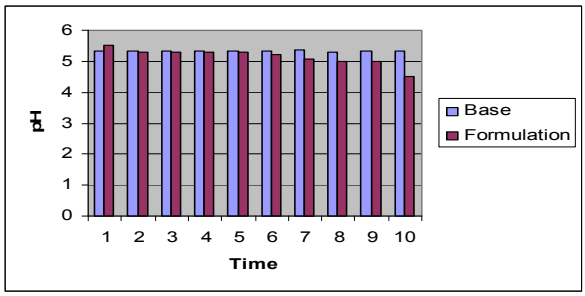

Figure 3. pH Values of Base and Formulation Kept at 40°C. Here 1=0 Hour, 2 =12 Hour, 3=24 Hour, 4=36 Hour, 5=48 Hour, 6=72 Hour, 7=7 Days, 8=14 Days, 9=21 Days, 10=28 Days.

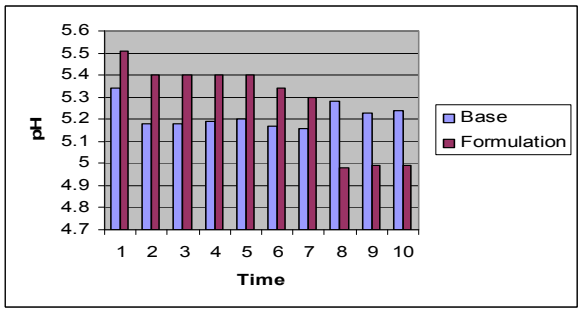

Figure 4. pH Values of Base and Formulation Kept at 40°C +75% RH. Here 1=0 Hour, 2 =12 Hour, 3=24 Hour, 4=36 Hour, 5=48 Hour, 6=72 Hour, 7=7 Days, 8=14 Days, 9=21 Days, 10=28 Days.

H. *Patch Test*

In this study patch tests were performed on forearms of volunteers for 24 hours for both the formulation and base, to check the safety of the formulation and the base on human skin. It was found that erythema level was decreased after the application of base and formulation by the end of 24 hours and no skin irritation was produced by the two creams after 24 hours as represented in **fig. 5 & 6.** With paired sample t-test it was evident that the effects of formulation and base were insignificant regarding the skin erythema.

It is concluded that the formulation and the base produced no skin irritation after performing patch test of 24 hours. It may be attributed to the presence of a good emollient glycerin in the base and formulation, and/or Glycyrrhiza glabra natural antioxidant in an extract of glycyrrhiza glabra [22], in the formulation, which has the ability to reduce skin erythema [23].

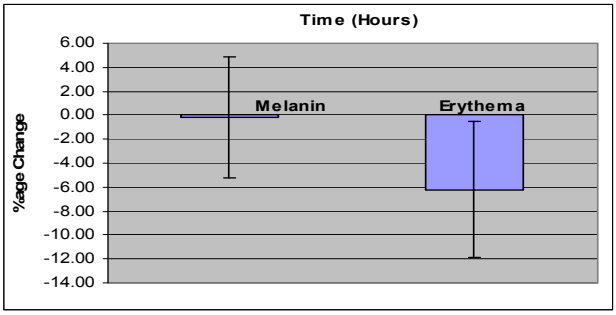

Figure 5. Percentage of Change in Melanin/Erythema in case of Base after 24 Hours

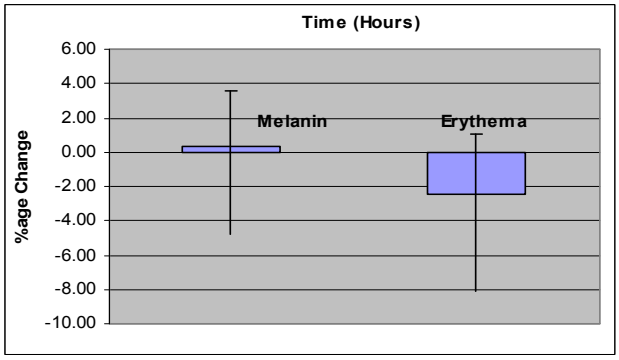

Figure 6. Percentage of Change in Melanin/Erythema in case of Formulation after 24 Hours

I. *Skin Melanin*

In this study, the effect of the base and the formulation on the production of skin melanin was examined. The amount of melanin was measured for 4 weeks at different time intervals in each individual after application of base & formulation and it was found that the base and formulation decreased the melanin contents in the skin till the end of 28 days but this decrease in melanin was more pronounced after the application of formulation as compared to base as shown in **fig.7, 8.** With the help of ANOVA test it was found that the base produced insignificant effects on skin melanin content throughout the study period of four weeks and formulation produced significant effects on skin melanin content in volunteers. By applying LSD, it was found that the formulation produced significant effects on skin melanin from 2nd to 4th week of study period. With the help of paired sample t-test it is evident that an insignificant difference was produced between the melanin effects of base and the formulation from 1st to 4th week of study period.

This showed that the two creams, the formulation and the base, have different effects on melanin. It is concluded that the decreased skin melanin content after application of formulation may be attributed to the tyrosinase inhibitory activity of Glycyrrhiza glabra extract [8]. Also the antioxidants present in extract may contribute to decrease in skin melanin content [10].

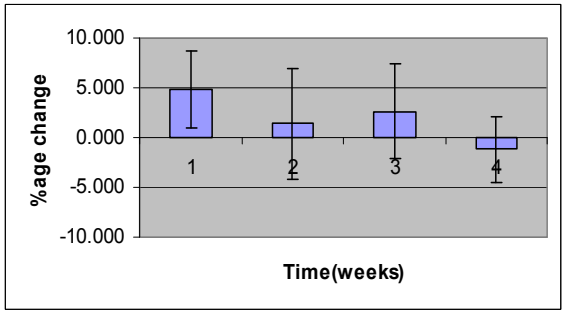

Figure 7. Percentage of Change in Skin Melanin Content after Application of Base for 4 weeks.

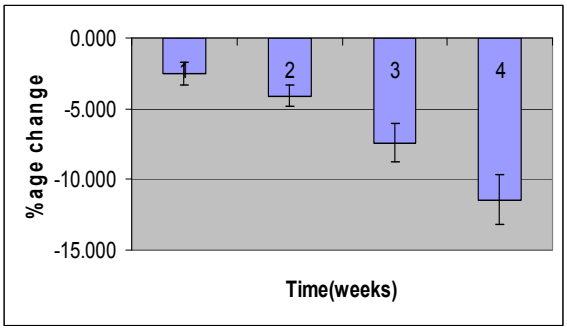

Figure 8. Percentage of Change in Skin Melanin Content after Application of Formulation for 4 weeks.

J. *Erythema*

For confirming the safety of cosmetics, the important point is that cosmetics must not cause any contact dermatitis when applied to the skin. The cause of contact dermatitis is not always due to cosmetic ingredients. Even if the safety of cosmetics is verified, it is known that environmental conditions such as temperature and humidity, misuse by the consumer, and the physical conditions may all cause contact dermatitis. Skin irritation is caused by the direct toxicity of chemicals on cells or blood vessels in the skin and is different from contact allergy which is caused by immune response [24].

In this study it was found that erythema contents were decreased from 1st to 4th week after the application of base and formulation as shown in **fig.9 & 10**. With the help of ANOVA test it was found that the base and formulation produced significant effects on skin erythema at different time intervals and by applying LSD in case of Base and formulation there was significant effect from 1st to 4th week of study period. With the help of paired sample t-test it was evident that there was insignificant variation in irritation with respect to base & formulation throughout the study period.

It is concluded that the decreases in erythema contents of skin at the end of study period after the application of base is due to presence of coconut oil in base which is a good emollient [25] and decrease inflammation [26]. Also the decrease in erythema content after application of formulation is due to the presence of Glycyrrhiza glabra extract which soothe and calm the skin [27].

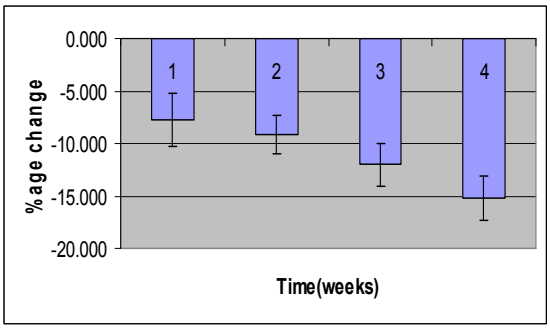

Figure 9. Percentage of Change in Skin erythema after Application of Base for 4 weeks.

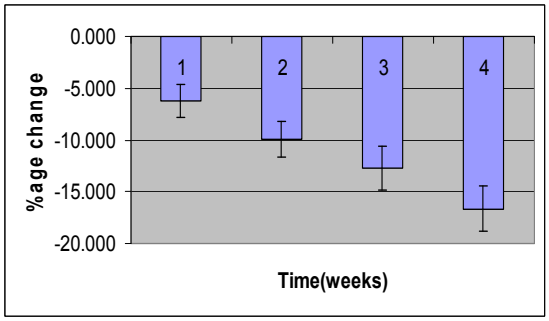

Figure 10. Percentage of Change in Skin erythema after Application of Formulation for 4 weeks.

ACKNOWLEDGMENT

The authors thanks to Higher Education Commission of Pakistan for financial support and The Department of Pharmacy, The Islamia University of Bahawalpur for moral support.

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