

*Full Length Research Paper*

## Assessment of anti erythmic and skin whitening effects of milk thistle extract

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**This study was intended to explore the effects of a topical cream (w/o emulsion) of milk thistle (*Silybum marianum*) extract against its vehicle as control on skin erythma and skin melanin. Milk thistle extract was entrapped in the inner aqueous phase of w/o emulsion. The vehicle (base without extract) and the active formulation (containing 4% extract) were applied by volunteers (n = 11) for a period of 12 weeks. Skin erythma and melanin were measured fortnightly to determine any effect produced by these topical creams. Base showed significant ( $P \leq 0.05$ ) increase while active formulation showed significant decrease in skin melanin content. In the case of skin erythma, the base showed insignificant decrease ( $P \geq 0.05$ ), whereas the formulation showed statistically significant decrease. The study showed that active formulation containing extract of milk thistle reduced skin melanin due to the presence of silymarin flavonoids. The formulation also possessed anti-inflammatory effects and was found to be suitable for topical application.**

**Key words:** *Silybum marianum*, w/o emulsion, anti-erythmic, skin whitening, mexameter.

### INTRODUCTION

Human skin exists in a wide range of different colors and gradations, ranging from white to brown to black. This is due to the presence of a chemically inert and stable pigment known as melanin, which is produced deep inside the skin (Costin and Hearing, 2007). Melanin determines the phenotypic appearance of humans and plays an important role in protecting human skin from the detrimental effects of ultra violet (UV) sun radiation and in scavenging toxic drugs and chemicals (Choi et al., 2002). The accumulation of an irregular melanin quantity in the skin might become an esthetic problem. Increased melanin is an extremely anxiety-producing symptom, and it can be a source of significant emotional disturbances for individuals, especially for women (Amer and Metwalli, 2000). In western countries, skin lighteners are applied for the prevention and treatment of irregular pigmentation such as melasma, freckles or age spots. In Asia, the need

to make the skin whiter is widely extended by traditional beliefs (Solano et al., 2006).

In pharmaceutical, medicinal and cosmetic field, pigmentation has become an important phenotypical characteristic. Naturally and synthetic chemical agents can frequently modulate the metabolism of the pigmentation produced. In individuals with a dark skin complexion, alterations in skin pigmentation may often have a dramatic expression (Stratigos and Katsambas, 2004). As of increasing focus on skin appearance, many cosmetic and pharmaceutical companies are focusing on research that will alter skin pigmentation. There are many known substances that can reduce the level of pigmentation in the skin (Gillbro and Olsson, 2011).

Medicinal plants are most suitable for pharmacological research and drug development, because their constituents can be used not only as therapeutic agents but also as starting materials or models for the synthesis of drugs or pharmacologically active compounds (Zhong et al., 2006). Milk thistle (*Silybum marianum*) from Asteraceae plant family, is an edible plant, native to the Mediterranean region of Europe but also widely dispersed

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to many countries throughout the world (Fraschini et al., 2002). It is a well known medicinal plant that has been used for centuries as a herbal medicine for the treatment of liver-related diseases. It is widely prescribed and has no side effects (Dixit et al., 2007).

In this study, w/o emulsion containing extract of milk thistle was evaluated for effects on diverse parameters related to skin.

## MATERIALS AND METHODS

Mexameter<sup>®</sup> MPA 5 (Courage Khazaka, Germany), Abil-EM90<sup>®</sup> (Franken Chemical, Germany), 95% Ethanol (BDH Chemicals, England) and Paraffin oil (Merk KGaA Darmstadt, Germany). Milk thistle seeds (World Homeo & Herbal Pharma<sup>®</sup>, Islamabad, Pakistan). Distilled water (Department of Pharmacy, Islamia University of Bahawalpur, Pakistan).

Milk thistle seeds were identified at Cholistan Institute of Desert Studies, Islamia University of Bahawalpur and a voucher specimen was preserved (voucher # MT-SD-4-11-19) at the herbarium for future reference. Shinoda test was performed for the detection of flavonoids. Few magnesium turnings and concentrated hydrochloric acid were added to the test solution. After few minutes the colour of the solution turned crimson red indicating the presence of flavonoids in the extract (Prabhu et al., 2011).

### Tested formulations

The tested formulations in this study were w/o emulsions (base and formulation). These were previously prepared by adding aqueous phase to the oily phase with continuous stirring. Oil phase comprised of paraffin oil and surfactant (Abil<sup>®</sup>-EM 90), and aqueous phase comprising of water was heated to  $75 \pm 1^\circ\text{C}$  and then extract was added in it. In the case of base, no extract was added in the aqueous phase. The creams were named A (active formulation having extract) and B (base with no extract).

### Assessment of skin irritation

To assess any skin irritation, patch tests were performed on both forearms of each volunteer on the first day of skin testing. A  $5 \times 4$  cm region was marked on the forearms. The patch (Bandage disc) for the left forearm was saturated with 1.0 g of base while the patch for right forearm was saturated with 1.0 g of formulation. Each was applied to the marked regions separately on each forearm. The regions were covered with the surgical dressing after application. The patches were removed after 48 h and the forearms were observed for any skin irritation by a physician (Hachem et al., 2002).

### Study protocol

Eleven male volunteers having no skin or other disease with mean age of 46 years were selected for the study and consent forms were taken. The study was designed single blinded for the comparison of two creams. The experiments were carried out on the cheeks of volunteers as cheeks are more susceptible to UV radiations. Every volunteer applied creams at night on cheeks for the period of 12 weeks and came for measurement on 2nd, 4th, 6th, 8th, 10th and 12th week in the morning at 10 a.m. They were allowed to wash their faces with water and sit to become accustomed with the environment for 30 min before any measurements were

taken. Values for different parameters were taken in controlled room temperature  $25 \pm 1^\circ\text{C}$ . Every volunteer was provided with a questionnaire at the end of the study to test the sensory values of creams. The questionnaire consisted of seven parameters to be evaluated and every parameter was assigned 11 values from -5 to +5 indicating very bad to very good, respectively. The parameters were 1, ease of application; 2, spread ability; 3, sense just after application; 4, sense in long term; 5, irritation; 6, shine on skin; 7, sense of softness.

### Ethical standards

This study was approved by the Board of Advanced Studies and Research (BASR), and its Ethical Committee for *In-vivo* Studies (Reference No 3715/Acad.), Islamia University of Bahawalpur and was conducted according to the international guidelines of Helsinki Declaration (Bosnjak, 2001).

### Mathematical analysis

The percentage changes for the individual values of skin erythma and melanin of volunteers were calculated by the following formula:

$$\text{Percentage change} = [(A - B)/B] \times 100 \quad (1)$$

Where A is the individual value of any parameter of 2nd, 4th, 6th, 8th, 10th or 12th week and B is the zero hour value of that parameter.

### Statistical analysis

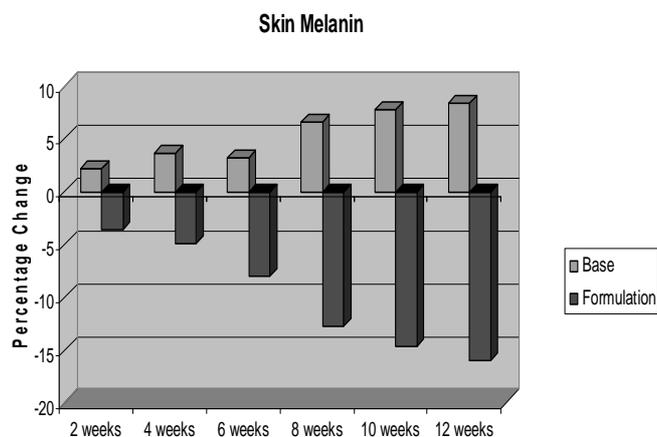
Paired samples t-test for variation between the two preparations and two-way ANOVA for variation between different time intervals were analyzed using SPSS 12.0 on computer using a 5% level of significance.

## RESULTS AND DISCUSSION

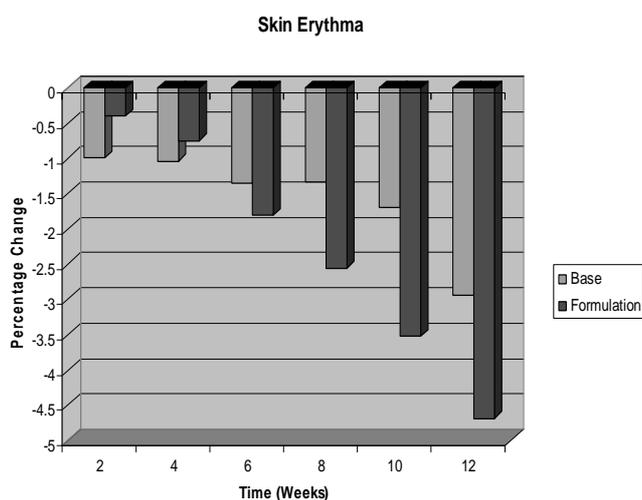
### Skin melanin

In this study, it was found that there were increase in skin melanin values after the application of base, but in the case of formulation there was continuous decrease in skin melanin content throughout the study period. Skin melanin was determined at regular intervals of 2nd, 4th, 6th, 8th, 10th and 12th week and percentage of changes are represented in Figure 1. With the help of ANOVA test, it was found that changes in skin melanin values produced by base and the formulation was significant ( $P \leq 0.05$ ) with respect to time. By applying paired sample t-test, it was found that formulation produced significant ( $P \leq 0.05$ ) effects with respect to base.

The decline in skin melanin can be credited to the polyphenolic flavanoids present in milk thistle which include silymarin comprising of silibinin, silydianine, silychristine and other flavonolignans (Kshirsagar et al., 2009). Tyrosinase is the enzyme responsible for melanin biosynthesis. Silymarin significantly prevent melanin production in melanocyte cells through inhibition of



**Figure 1.** Percentage of changes in skin melanin after application of base and formulation.



**Figure 2.** Percentage of changes in skin erythma after application of base and formulation.

tyrosinase expression due to which skin whitening effects were observed (Choo et al., 2009).

### Skin erythma

Patch test indicated that both base and formulation did not irritate the skin. In this study, it was found that there was a regular decrease in skin erythma throughout the study period after the application of base samples as well as active formulation. There was gradual decrease in skin erythma throughout the study period. The percentage changes determined after 2nd, 4th, 6th, 8th, 10th and 12th week and represented in Figure 2 show that there was more decline in erythma exhibited by the formulation as compared to base.

However, by applying ANOVA test, it was found that the base produced insignificant ( $P \geq 0.05$ ) effects on skin erythma with respect to time while formulation produced significant ( $P \leq 0.05$ ) effects on skin erythma with respect to time. Significant differences were observed between the skin erythma of base and the formulation when applying paired sample t-test.

Topical applications must not produce any irritation or redness. The decrease in skin erythma indicates that the formulation tends to soothe the skin and it can be due to flavonoids present in milk thistle that possess anti-inflammatory properties (Juma'a et al., 2009).

### Assessment of effectiveness of formulations

To assess the effectiveness of the two formulations, that is, base and active formulation, tested in this study, the volunteers were asked to answer a questionnaire consisting of seven parameters. Average points for the first parameter 'ease of application' of creams were found to be 4.60 and 4.80 for the base and formulation, respectively indicating that base and formulation can be easily applied on the skin. Average points for 'feel on application' were 4.10 for the base and 4.50 for formulation which indicated that formulation was felt well on the skin than base. 'Spread-ability' got 4.30 for base and 4.60 for formulation which meant that the formulation spread on skin better than the base. Average points for the 'sense on long-term application' of creams were 3.80 and 4.00 for the base and formulation, respectively. 'Irritation' got 0.00 point for both base and formulation. 'Softness of the skin' got 4.60 for base and 4.80 for formulation. Points for 'shine on skin' were 4.40 for the base and 4.70 for formulation. From paired sample t-test, non-significant difference between the average points for base and formulation were observed which showed that there was no variation between base and formulation regarding the effectiveness from sensory and cosmetic quality point of view.

### Conclusion

From this study, it can be concluded that milk thistle (*S. marianum*) possesses skin whitening properties as it significantly decreased skin melanin level when applied topically in the form of a w/o emulsion. In addition, decrease in skin erythma/redness showed that the formulation has anti-inflammatory effects. Evaluation for effectiveness by a questionnaire suggested that it can be used as cost effective topical skin whitening treatment as the formulation exert no harmful effects.

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