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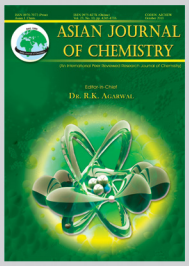

Cosmetic Application of Phenolic Cream from Mulberry Bark Extract

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The aim of this study is to determine the effects of newly formulated O/W emulsion (cream) of ethanolic mulberry extract *versus* its vehicle (base) as control on skin melanin, skin erythema and skin moisture content. Concentrated 4 % mulberry extract was entrapped in the inner aqueous phase of O/W emulsion. The newly formulated base and formulation were applied to the cheeks of undiseased human volunteers for a period of 8 weeks. Melanin, erythema and skin moisture were monitored every week and analyzed by Mexameter and Corneometer. Statistically the results of both base and formulation were compared by using ANOVA and Paired *t*-test. The base showed insignificant ($p > 0.05$) effects while the formulation showed statistically significant ($p \leq 0.05$) decrease in melanin content. Both the base and formulation showed insignificant ($p > 0.05$) effects on skin moisture content. Skin erythema was significantly ($p \leq 0.05$) reduced by the formulation. This formulation of cream containing 4 % concentrated extract of mulberry can be used for skin whitening as it decreased skin melanin content. Furthermore, the decrease in erythema demonstrated that the cream will be safe to use and will not cause any irritation. The formulation is suggested not to be used in cold season as it causes dryness.

Key Words: Mulberry extract, Melanin, Erythema, Mexameter, Corneometer.

INTRODUCTION

Emulsion consists of two immiscible liquids. One phase is hydrophilic and the other phase is lipophilic. The base of the hydrophilic phases is usually water or a fluid that is miscible with water. It is thus known as the water phase (W). The lipophilic component of an emulsion can be a fat, oil, mineral oil, or other organic fluid. This is generally known as the oil phase (O). In an emulsion, one immiscible liquid is dispersed in the other form a dispersion medium in which various states of distribution are possible depending on which component forms the dispersed phase and which forms the continuous phase[1]. The system being stabilized by the presence of a third substances, the emulsifying agent[2]. Emulsion products are playing an important role in variety of industry fields such as foodstuffs, cosmetics, pharmaceuticals and cleaning agents, lubricants and so on. The emulsions cream prepared by homogenizing oil and aqueous phases are thermodynamically unstable and are subject to phase separation with time because of the large difference in density between the oil and water[3]. Particularly advantageous cosmetic emulsion preparations are obtained when antioxidants are used as active ingredients. There is a growing interest in natural antioxidants found in plants. Many antioxidants are acting compounds which are isolated from natural herbs and plants (extracts) and are used as potential antioxidants in cosmetics.

An extract of mulberry is rich in natural antioxidants. Mulberry has high phenolic compounds which have high levels of total anthocyanin[4]. Anthocyanins (Fig. 1) are considered good antioxidant agents. Anthocyanins, gallic acid, flavonoids and tannins in preventing cell damage make them the strongest candidates for cosmetic application[5]. Also the prsesnce of tyrosinase inhibitors activities and its free radical scavenging role, are important in cosmetic skin-whitening[6,7]. Oxidative stress contributes to skin aging and can adversely affect skin health. Antioxidants active in skin cells may support skin health[7]. Morus alba has been reported to show tyrosinase inhibition and inhibitory effects on tyrosinase activity and melanin formation in B-16 melanoma cells[6]. This herbal medicine has been used as a cosmetic additive as antiaging and skin-whitening[5].

Mulberry extract is water miscible and can be incorporated in the aqueous phase of oil in water. Mulberry can be used for hypertension, antihypoglycemic, antibacterial activity and antiviral activity. Moreever it can be used as antiinflammatory, anthelmintic, laxative and as food source for silkworm[8,9].

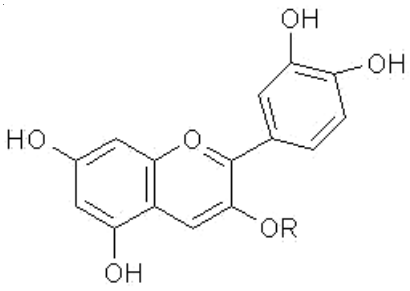

Fig. 1. Structure of Anthocyanin (a phenolic compound) found abundant in mulberry[6]

Paraffin oil was used which consists chiefly of a mixture of hydrocarbons belonging to the methane series. It occurs as a colourless, oily, transparent, tasteless, non-fluorescent liquid, odorless when cold, but having a faint petroleum odor when heated[10].

EXPERIMENTAL

For the formulation of emulsions/creams (applied in the study) mulberry root barks were purchased from local market of Pakistan. Paraffin oil and stearic acid were obtained from Merck (Germany). Emulsifying Wax BP and lemon oil were purchased from local market (Pakistan).

Mexa meter MPA5 (Courage + Khazaka, Germany), Corneometer MPA5 (Courage + Khazaka, Germany), Digital Humidity Meter (TES Electronic Corp, Taiwan), Electrical Balance (Precisa BJ-210, Switzerland), Homogenizer (Euro-Star, IKA D 230, Germany), pH-Meter (WTW pH-197i, Germany), Rotary evaporator (Eyela, Co. Ltd. Japan), Water Bath (HH .S21 4., China), Hot Incubators (Sanyo MIR-162 and 153, Japan).

Identification of plant: The identification of mulberry was performed by Dr. Arshad at Cholistan Institute of Desert Studies, The Islamia University of Bahawalpur, Pakistan. The specimen was deposited in the pharmacognosy section of faculty of pharmacy and alternative medicine. The voucher number is: pharm.07/Feb 2007.

Methods

Preparation of mulberry extract: An extract of mulberry was obtained by macerating the fine powder of peeled root bark, shade dried and grounded to have coarse powder. The fine powder was placed in hydro alcoholic mixture, then filtering and concentrating it on rotary evaporator. The extract was preserved in freezer at 0 ºC.

Preparation of formulation and base: In this study, O/W emulsion was prepared by the addition of aqueous phase to the oily phase with continuous agitation. Oily phase that consisted of paraffin oil, stearic acid and surfactant (emulsifying wax BP) was heated up to 75 ±1 ºC. At the same time, aqueous phase consisting of water was heated to the same temperature and then the mulberry extract was added in it. 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 15 min until complete aqueous phase was added, 2-3 drops of lemon oil were added during this stirring time to give good fragrance to the formulation. After the complete addition of the aqueous phase, the speed of the mixer was reduced to 1000 rpm for homogenization, for a period of 5 min and then the speed of the mixer was further reduced to 500 rpm for 5 min for complete homogenization; until the emulsion cooled at room temperature[11].

The base was prepared with the same method as above; the only difference was the absence of Mulberry extract *i.e.*, the active constituent in the aqueous phase.

Study protocol for skin evaluation: Eleven male volunteers were selected with ages between 25 and 45 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 25 ºC 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 creams. 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 twice at night to the respective cheek. The creams were applied by the volunteers themselves as instructed for 60 days. Every individual was instructed to come on days 7, 14, 21, 28, 42 and 56 for the skin measurements.

Ethical standards: This study was approved by the Board of advance study and Research (BASR), The Islamia University of Bahawalpur and institutional ethical committee in compliance with NIH Principles of Laboratory Animal Care 1985. The Reference No is COSM -3987/07.

Statistical analysis: The measured values obtained for different parameters (skin moisture, melanin, erythema) were analyzed using SPSS 12.0 on the Personal computer (paired samples *t*-test for variation between the two preparations; two-way ANOVA for variation between different time intervals and different individuals).

Mathematical analysis: The percentage changes for the individual values of different parameters, taken every week, of volunteers were calculated by the following formula;

$$\text{Change } (\%) = \left[\frac{(A - B)}{B}\right] \times 100 \quad (1)$$

where; A = individual value of any parameter of 1st, 2nd, 3rd, or 4th week, B = zero hour value of that parameter.

RESULTS AND DISCUSSION

Melanin and erythema content: Skin erythema and skin melanin content were measured before application of creams (zero hour readings) and then at 1st, 2nd, 3rd, 4th, 6th and 8th week of study period by Mexameter MPA 5 (Courage and Khazaka GmbH). The per cent changes occurred in the values for 11 volunteers were calculated by eqn. 1 (Figs. 2 and 3).

Skin moisture content: Skin moisture content was measured before application of creams (zero hour readings) and then at 1st, 2nd, 3rd, 4th, 6th and 8th week of study period by

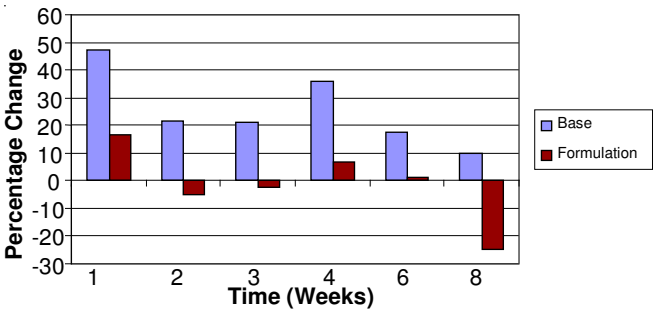

Fig. 2. Percentage of change in skin melanin content after application of base and formulation

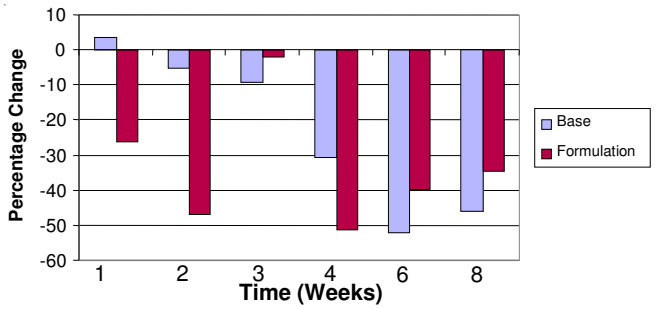

Fig. 3. Percentage of change in skin erythema content after application of base and formulation

Corneometer MPA 5 (Courage and Khazaka GmbH). The per cent changes occurred in the values for 11 volunteers were calculated by eqn. 1 (Fig. 4).

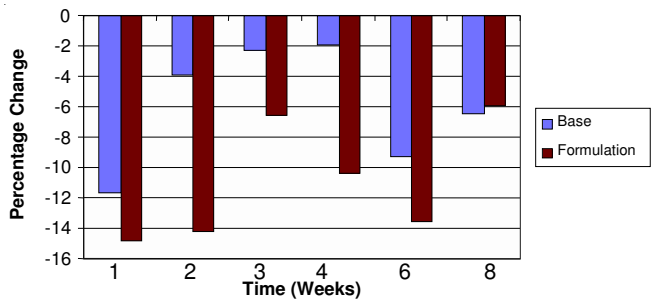

Fig. 4. Percentage of change in skin moisture content after application of base and formulation

Melanin: In this study, the effect of the base and formulation on the production of skin melanin was examined. The amount of melanin was measured for 8 weeks at different time intervals in each individual after application of base and formulation. It was found that the base increased the melanin contents in the skin till the end of 8 weeks while the formulation decreased the melanin contents till the end of study period as shown in Fig. 2.

With the help of ANOVA test it was found that the changes in melanin produced by base were insignificant ($p > 0.05$) while the changes in melanin by formulation were significant ($p \leq 0.05$). LSD test showed that base produced insignificant effects throughout the study period while formulation showed significant effects at 1st, 2nd, 3rd and 4th week. With the help of paired sample *t*-test it was concluded that a significant difference was observed between the melanin effects of base and formulation till the end of study period.

This showed that the two creams, the formulation and the base have different effects on melanin. It was concluded that the decreased skin melanin contents after application of formulation may be attributed to the presence of phenolic compounds *i.e.*, anthocyanin and flavonoids, which has tyrosinase inhibitor activity useful for skin-whitening[6].

Erythema: In this study, erythema was constantly monitored every week for the base and the formulation throughout the period of application. It was found that erythema contents were slightly increased at 1st week after the application of base and gradually decreased from the 2nd week till 8th week of study period. Whereas after the application of formulation erythema contents were continuously decreased from 1st week to 8th week of study period as shown in Fig. 3.

With the help of ANOVA test it was found that the base and formulation produced significant ($p \leq 0.05$) effects on skin erythema at different time intervals. By applying LSD test it was seen that base produced significant effects at 6th and 8th week while on the other hand formulation showed significant effects at, 2nd, 4th, 6th and 8th week of study period. With the help of paired sample *t*-test it was evident that there was significant variation in irritation with respect to base and formulation at 4th and 6th week.

It is concluded that the formulation decreased the erythema contents of skin at the end of study period and overall effect of formulation on skin erythema was significant, so it can be used safely without any skin irritation. This is in accordance with literature as the mulberry extract is a good source of ascorbic acid and flavonoids having antinflammatory activities[12].

Skin moisture content: The moisturization of skin involves repairing the skin barrier, retaining/increasing water content, reducing TEWL, restoring the lipid barriers' ability to attract, hold and redistribute water and maintaining skin integrity and appearance. Mulberry has different active ingredients like citric acid, vitamin C, palmitic acid and anthocyanins[4] which has ability in hydration conditions. Skin needs lipids and water. When deprived of its natural hydration factors, the skin tends to become dehydrate and discoloured and lose its elasticity[13]. Reduction in the water contents of skin can lead to changes in the skin's viscoelasticity. Presence of anioinc surfactants like sodium lauryl sulphate is able to interact directly with biological membranes and remove lipids from intercellular spaces of the stratum corneum and leads to dehydration.

In this study, it was found that there was a decrease in moisture values from 1st week to 8th week after the application of the base and the formulation as shown in Fig. 4.

With the help of ANOVA test it was found that both the base and formulation showed insignificant ($p > 0.05$) variation throughout the study period. By LSD test it was found that significant change in moisture content was observed at 1st week after application of base and at 1st, 2nd, 4th and 6th week after application of formulation. With the help of paired sample *t*-test it was evident that a significant difference in the moisture values was produced at the 6th week when base was compared with formulation. The reasons for the reduction of water contents may be attributed to the presence of sodium lauryl sulphate in emulsifying wax which reacts with the lipids

of skin and removes them so lipid barrier is disturbed leading to more water loss from the skin.

Conclusion

A stable cream containing 4 % concentrated ethanolic extract of mulberry can be formulated with emulsifying wax. This cream decreased the skin melanin content and erythema contents within a study period of 8 weeks indicating bleaching effects with safety. The cream also showed skin drying effects as it causes a reduction in skin moisture content indicating the cream can be used for acne however a targeted study is suggested in future.

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