



Development of Skin-friendly Dermatological Water-in-Oil Emulsion of Pomegranate Juice

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Abstract: This study was designed to develop a topical skin-care cream (w/o emulsion) of 4% pomegranate extracts versus its vehicle (the base) as control and evaluate its effects on skin-melanin, skin erythema, skin moisture, skin sebum and TEWL. Concentrated pomegranate (*Punica granatum*) juice was entrapped in the inner aqueous phase of w/o emulsion. The base containing no extract and a the formulation containing 4% concentrated juice of pomegranate was formulated. The odour was adjusted with few drops of lemon oil. Both the base and the formulation were stored at different storage conditions for a period of four weeks to predict their stability. The stability parameters, i.e., physical stability, centrifugation and pH, were monitored at different time intervals. Both the base and the formulation were applied to the cheeks of 25 healthy human volunteers for a period of 8 weeks. The pharmaceutical stability of creams was achieved from 4 weeks in-vitro study period. Odour disappeared with the passage of time due to volatilization of lemon oil. The formulation exhibited significant ($P<0.05$) effects on skin melanin and skin erythema. Both the vehicle (base) and the formulation tended to increase sebum content. The results showed a good stability over 4 weeks in-vitro observation period of both the base and the formulation and the formulation exhibited bleaching, anti-inflammatory and moisturizing effects. Both the base and the formulation were elegant and aesthetic with respect to sensory evaluation.

Keywords: Antioxidant, emulsion, *Punica granatum* extract, skin, TEWL

1. INTRODUCTION

Punica granatum (Punicaceae) is a symbol of life, longevity, health, femininity, fecundity, knowledge, morality, immortality and spirituality. In Ayurvedic medicine *Punica granatum* is considered “*A Pharmacy unto Itself*” [1]. This herb was used in ancient Egypt for treatment of inflammation of the skin, mucosa and joints. The major constituents are tannins (25–28%) including punicalagin; polyphenols such as ellagic acid, ascorbic acid, niacin, potassium and piperidine alkaloids. *Punica granatum* functions as an astringent. It has documented antimicrobial activity for gram-

negative bacteria, *Saccharomyces* fungus, parasites and viruses [2].

Pomegranate juice comprises of 85% water, 10% carbohydrates, and 1.5% fiber tissues, vitamin C, antioxidants and coloring material [3]. Pomegranate is the potent source of polyphenolic compounds, i.e., flavonoids and hydrolysable tannins (punicalin, pedunculagin, punicalagin, gallic acid and ellagic acid) which have 92% radical scavenging activity [4, 5]. It is a rich source of polyphenolics, which have shown anti-inflammatory, antioxidant, and anticarcinogenic activity in numerous in vivo and in vitro studies [6]. This application relates to

dermatological agents for treating dermatological disorders. The dermatological agents include a therapeutically effective amount of at least one *Punica granatum* fruit extract in an amount sufficient to neutralize free radicals, a moisturizing agent in an amount sufficient to facilitate hydration of the skin, and a pharmaceutically acceptable carrier [7]. Pomegranate extract is water miscible and is incorporated in the aqueous phase of water-in-oil emulsion. The skin lightening effects of ellagic acid may be due to chelating copper at the active site of tyrosinase. Skin whitening, moisturizing, sun protection and anti-wrinkle, prevent and treat the acne, anti-stain and anti-freckles effects are mainly attributed to ellagic acid [8, 9]. An emulsion is a biphasic system consisting of two immiscible liquids one of which (the dispersed phase) is finely and uniformly dispersed as globules throughout the other phase (the continuous phase) [10]. The main advantage of emulsions is that they increase the solubility and bioavailability of therapeutic drugs as well as the ability to favor the topical transport of hydrophilic solute. It has been shown that microemulsions can be formed spontaneously and are thermodynamically stable, on one hand they improve drug solubilization and bioavailability and on the other hand they act as potential drug delivery systems by integrating a wide range of drug molecules. The main advantage of using the topical emulsions is to avoid gastrointestinal environment and first pass effect [11]. ABIL EM 90 is a lipophilic surfactant which is chemically cetyl dimethicone copolyol [12]. ABILE EM90 is suitable for the formulation of water-in-oil emulsions, w/o/w and o/w/o multiple emulsions. Preparations with ABIL EM90 are highly stable towards heat [13]. Human skin has 10-20% water content and its hydration level can be enhanced by topical applied hydrating cosmetics. Skin is viscoelastic in nature due to collagen and fibers. Skin hydration and skin lipid contents improve skin elasticity [14].

2. MATERIALS AND METHODS

2.1 Ingredients

Pomegranate fruit was used as a plant material. Extract of *Punica granatum* (purchased locally) was prepared in lab of Pharmacy Department, The

Islamia University of Bahawalpur, Pakistan. ABIL-EM90 was purchased from Franken Chemical (Germany) and paraffin oil was taken from Merk KGaA Darmstadt (Germany), while Distilled Water was prepared in labs of Pharmacy Department, The Islamia University of Bahawalpur, Pakistan.

The identification of *Punica granatum* (Family: *Punicaceae*) was performed in Cholistan Institute of Desert Studies (CIDS) at The Islamia University of Bahawalpur, Pakistan. The specimen was deposited in the herbarium; the Voucher number is: *P. granatum* 7072/21-04-1925 Rawal Chand Herbarium of Department of Botany, University of Agriculture, Faisalabad, Pakistan.

The free radical scavenging activity of *Punica granatum* was determined in accordance to Kulkarni AP with slight modification using DPPH (1, 1-diphenyl-2-picrylhydrazyl) which is a stable free radical [15]. Fresh extract of *Punica granatum* (1 mL) was mixed with 100 mM Tris-HCl (9 mL) buffer with a pH of 7.4. One mL of DPPH (500 μ M in distilled water) was added to it. The obtained mixture was kept at room temperature for 20 minutes. Then, the absorption of the mixture at 517 nm was taken, in comparison with the control solution (mixture without DPPH). The activity of free radicals was calculated in % inhibition according to the following relation:

$$\% \text{ Inhibition} = \frac{(A \text{ control} - A \text{ test})}{A \text{ control}} \times 100$$

A control

The antioxidant activity of *P. granatum* was observed to be 78%. The apparatus used were:

Centrifuge Machine, Hettich EBA 20, Germany;

Cold Incubator, Sanyo MIR-153, Japan

Conductivity-Meter, WTW COND-197i, Germany

Corneometer MPA 5, Courage + Khazaka, Germany

Mexameter MPA 5, Courage + Khazaka, Germany

Sebumeter MPA 5, Courage + Khazaka, Germany;

TEWA meter MPA 5, Courage + Khazaka, Germany

Digital Humidity Meter, TES Electronic Corp, Taiwan

Electrical Balance, Precisa BJ-210, Switzerland
Homogenizer, Euro-Star, IKA D 230, Germany
Hot Incubator, Sanyo MIR-162, Japan
PH-Meter, WTW pH-197i, Germany
Refrigerator, Dawlance, Pakistan
Rotary evaporator, Eyela, Co. Ltd. Japan SPSS,
12.0

2.2. Preparation of Extract

Extract of *Punica granatum* was obtained by concentrating the aqueous juice of pomegranate. The fruits were thoroughly rinsed with water before subjecting them to juice extraction. Thick-skinned pomegranate fruits were cut in halves prior to extraction. Juice extraction was carried out by using a mechanical juice extractor. Extracted juice, as came from the extractor was cloudy, containing pulp, fibers and seeds, so first, a fine screening was done by first passing the extracted juice from muslin cloth (16 layers) and then from filter paper under reduced pressure; for the complete removal of the seeds and pulp from the extract. Then, the quantity of extracted juice was measured. In order to maintain the quality of the extract, the container of juice was covered with aluminum foil and kept immediately in the refrigerator after it was removed from the extractor and screened. After screening the extract was then concentrated, bottled and frozen. pomegranate extract was concentrated to half of its original extracted amount; by just removing maximum part of its water. To prevent the loss of important compounds by heating, the extract was concentrated in rotary evaporator (Eyela, Japan) by providing low heat treatment, up to 45°C. Finally, the concentrated extract was packaged in glass tubes, sealed and frozen. Later, whenever required, the frozen extract of pomegranate was removed from the freezer, kept for sometime at room temperature to liquefy (i.e. freeze thawing) and used as an active ingredient in the aqueous phase of w/o emulsion [16].

2.3. Preparation of Emulsions

The W/O emulsions (base and formulation) were prepared by the addition of aqueous phase to the oily

phase with continuous agitation. For the preparation of formulation; oily phase that consisted of paraffin oil and surfactant (ABIL- EM 90) was heated up to $75\pm 1^\circ\text{C}$. At the same time, aqueous phase consisting of water was heated to the same temperature and then the *Punica granatum* 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 minutes until complete aqueous phase was added, 2 to 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 minutes and then the speed of the mixer was further reduced to 500 rpm for 5 minutes for complete homogenization; until the emulsion cooled to room temperature.

The base was also prepared by the above stated method using the same ingredients, excluding pomegranate extract (i.e., the active ingredient) [16].

Composition of the Base

Oily Phase: Paraffin oil, 16%; and Abil-EM 90, 4%.

Aqueous phase: Distilled water (q.s.), 100%.

Composition of the Formulation

Oily Phase: Paraffin Oil, 16%; and ABIL-EM 90, 4%.

Aqueous phase: Pomegranate extract, 4% (concentrated); and Distilled water (q.s.), 100%.

Both the creams were analyzed to assure the formulation of desired type. Emulsion was analyzed organoleptically (color, thickness, look, feel, liquefaction) and physically (creaming and phase separation).

Type of emulsion was analyzed by diluting the emulsion with oil and water separately.

pH value of freshly prepared emulsion and emulsions kept at different conditions were determined by a digital pH-Meter present in cosmetic laboratory.

Electrical conductivity of freshly prepared

emulsion and emulsions kept at different conditions were monitored by a digital conductivity-meter. Centrifugal tests were performed for emulsions immediately after preparation. The centrifugal tests were repeated for emulsions after 24hrs, 7, 14, 21 and 28 days of preparation. The centrifugal tests were performed at 25°C and at 5000 rpm for 10 minutes by placing the 5g of sample in disposable stoppered centrifugal tubes. Stability tests were performed at different conditions for emulsions to note the effect of these conditions on the storage of emulsions. These tests were performed on samples kept at 8°C ± 0.1°C (in refrigerator), 25°C±0.1°C (in incubator), 40°C±0.1°C (in incubator) and 40°C±0.1°C (in incubator) with 75% RH. Physical characteristic of simple emulsions, i.e. color, creaming and liquefaction, were noted at various intervals for 28 days.

One-sided blind study was designed with placebo control in the month of August to September (the moderate season in Pakistan). 25 healthy human volunteers who signed the informed consent, with age range 25-35 years were selected. Male volunteers were included in this work as they were easily available with regular under control observations. Prior to the tests, the volunteers were examined by a cosmetic expert for any serious skin disease or damage especially on cheeks and forearms. All the skin tests were performed at 21±01°C and 40±2% relative humidity conditions [14].

The experiments were carried out on the cheeks of volunteers as cheeks are uniformly and more prone to UV radiations. 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 60 days. Every individual was instructed to come on 1st, 2nd, 3rd, 4th, 6th and 8th week for the skin measurements.

2.4. Patch Tests (Burchard Tests)

On the first day of skin testing, patch tests were

performed on the both forearms of each volunteer. A 5cm X 4cm region was marked on the forearms. The patch (Bandage disc) for the right forearm was saturated with 1.0 g of base while the patch for left forearm was saturated with 1.0 g of 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. The patches were removed after 48 hours and the forearms were washed with physiological saline [16]. After 48 hours, scores were recorded for the presence of erythema (skin redness) using a scale with 4 points from 0 to 3.0 stands for absence of erythema, 1 for mild erythema, 2 for moderate erythema while 3 stands for severe erythema. Each volunteer was asked to note their irritation/itching towards the patches and then assign a score from the same scale. Average score with respect to volunteers is given in Fig. 1.

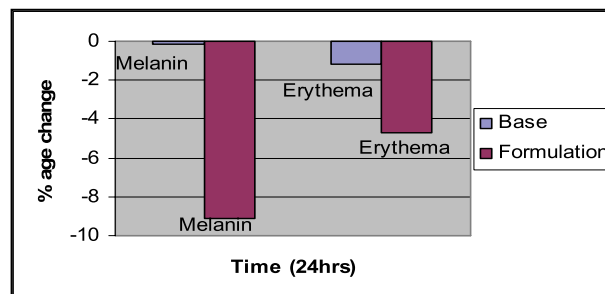


Fig. 1. Percentage change in Melanin/Erythema in case of the base and the formulation after 24 hours (Patch Test) Mexameter.

Mexameter was used to measure erythema and melanin on the cheeks of human volunteers, on the first day before the application of any cream and then on 1st, 2nd, 3rd, 4th, 6th and 8th week. With the help of a Corneometer, stratum corneum (SC) capacitance was measured before the application of any cream and then on 1st, 2nd, 3rd, 4th, 6th and 8th week. Sebumeter was used to measure sebum content of the skin before application of any cream and then on 1st, 2nd, 3rd, 4th, 6th and 8th week.

Every individual was provided with a form prepared previously to test the sensory values of creams. This form 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. This form was asked to be

completed independently by each individual on day 60.

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

$$\text{Percentage Change} = [(A - B) / B] * 100$$

where;

A = Individual value of any parameter (from 1st to 8th week)

B = Zero hour value of that parameter

The measured values obtained for different parameters (skin moisture, sebum, melanin, erythema, elasticity and pH) 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). A 5% level of significance was used ($\alpha=5\%$).

3. RESULTS AND DISCUSSION

3.1 Stability and Properties during Storage

Stability of the base and of the formulation kept at different storage conditions was studied and physical characteristics regarding the stability of the base and formulation have been discussed.

Centrifugation tests for the base and the formulation of freshly prepared creams and for samples kept at different storage conditions were performed and phase separation in these samples was observed for 28 days at different time intervals. No phase separation was observed in any sample of the base or the formulation throughout the study period. Electrical conductivity values of the base and formulation of fresh creams and samples kept at different storage conditions for 28 were also been determined. No electrical conductivity was noticed in any sample of the base and formulation throughout the study period. pH values of the base and the formulation of fresh creams and samples kept at different storage conditions up to 28 days have been determined and reported in Fig. 2. The percentages of changes in the amounts of erythema, melanin, skin moisture, skin sebum and transepidermal Water loss (TEWL) following the applications of

the base and formulation on the cheeks of human volunteers have been demonstrated in Fig. 3, 4, 5, 6 and 7, respectively.

Sensory evaluation of both the base and formulation by the volunteers has been presented in Fig. 8.

Both the base and the formulation were divided into to four samples separately and these samples were kept at different storage conditions i.e. at 8°C in refrigerator, at 25°C, 40°C and at 40°C + 75% relative humidity (RH) in stability chambers. These

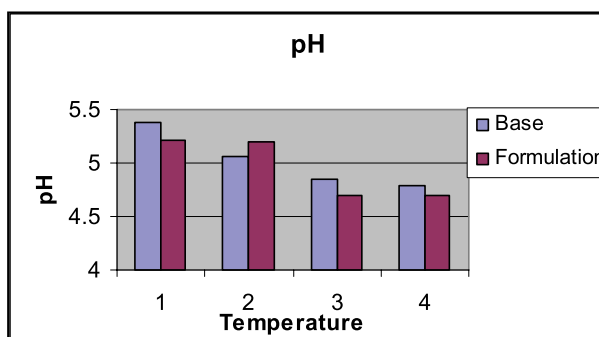


Fig. 2. pH of the base and the formulation kept at 8°C, 25°C, 40°C and 40°C+ 75% relative humidity.

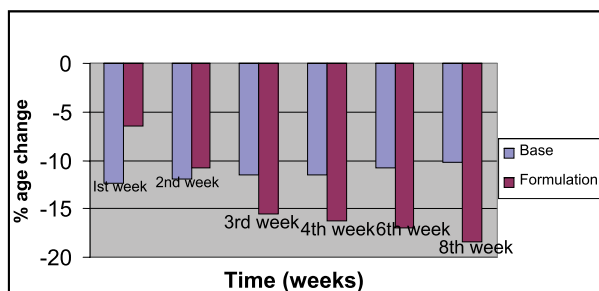


Fig. 3. Percentage change in skin melanin content after application of the base and formulation.

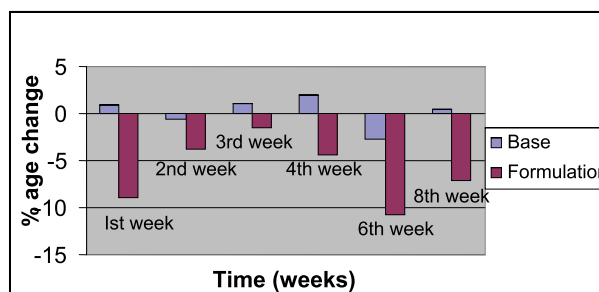


Fig. 4. Percentage change in skin erythema content after application of the base and the formulation.

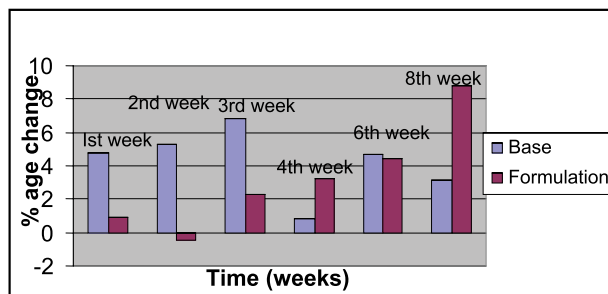


Fig. 5. Percentage change in skin moisture content after application of the base and formulation.

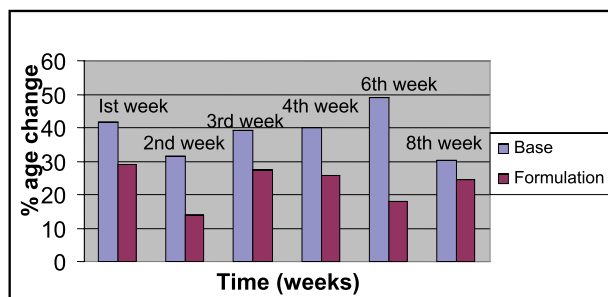


Fig. 6. Percentage of change in skin sebum after application of the base and formulation.

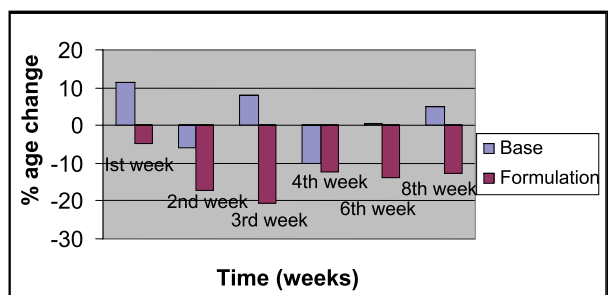


Fig. 7. Percentage change in trans-epidermal water loss (TEWL) after application of the base and the formulation.

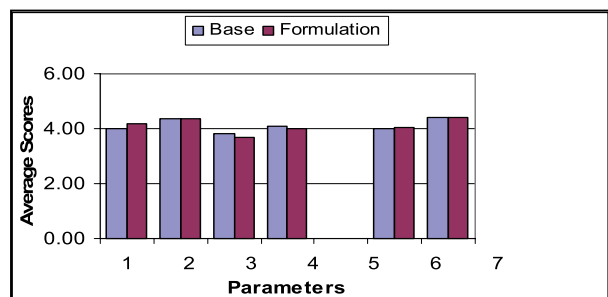


Fig. 8. Average results for panel test. (1= Ease of application, 2= Spreadability, 3= Sense just after application 4= Sense in long term, 5= Irritation, 6= Shine on skin, 7= Sense of softness)

samples of the base and formulation at different storage conditions were observed for a period of 28 days at different intervals with respect to change in color, liquefaction and phase separation.

The freshly prepared the base and formulation were creamy white in color. There was no change in color of any sample of the base or formulation at different storage conditions i.e., 8°C, 25°C, 40°C and the at 40°C+ 75% relative humidity up to the observation period of 28 days. Thus both the base and formulation were stable at the studied storage conditions up to 28 days.

No liquefaction was observed in any of the sample of the base and formulation kept at 8°C and 25°C during whole observation period of 28 days. A Slight liquefaction was observed in the sample of the base and formulation kept at 40°C and 40°C + 75% RH on 21st and 28th day of observation The samples of the base and formulation were stable at 8°C, 25°C, but slight phase separation in the sample of the base occurred at 40°C and 40°C+ 75% RH on 28th day of observation but the formulation was stable at 40°C and 40°C+ 75% RH on 28th day of observation. The centrifugation test was performed on the samples of the base and formulation kept at different storage conditions up to a period of 28 days at definite time intervals. No phase separation on centrifugation was seen in any of the samples kept at different storage conditions i.e. 8°C, 25°C, 40°C and 40°C+ 75% RH up to 28th day of observation. This indicated that the emulsions were stable at all the storage conditions for 28 days. Conductivity test was performed for all the samples of the base and the formulation kept at different storage conditions up to a period of 28 days at definite time intervals. No electrical conductivity was seen in any of the samples of the base and formulation kept at different storage conditions i.e. 8°C, 25°C, 40°C and 40°C+ 75%RH up to 28th day of observation. This indicated that creams were stable at different storage conditions.

pH of freshly prepared the base and formulation was 5.35 and 5.65 respectively, which is within the range of skin pH. The pH values of the samples of the base kept at different storage conditions i.e. 8°C, 25°C, 40°C and 40°C+ 75% RH was found to be increasing gradually in the 3 days and then it started

to decline continuously till 28th day with some variations. At the end of study pH of the samples of the base at 8°C, 25°C, 40°C and 40°C+ 75% RH was 4.52, 4.60, 4.35 and 4.08 respectively. Whereas pH of the samples of formulation kept at 8°C, 25°C, 40°C and 40°C + 75% RH showed gradual reduction in pH values with slight variations with time. The pH values of samples of formulation kept at 8°C, 25°C, 40°C and 40°C+ 75% RH were 4.60, 3.99, 3.39 and 4.01 at 28th day respectively. By using two-way analysis of variance at 5% level of significance, it was found that the change in pH of different samples of the base as well as formulation was significant ($p=002$ & $p=046$ respectively) at different levels of time and temperature. The decrease in pH of the formulation at different storage conditions might be due to the production of any acidic metabolite.

3.2. Dermatological Tests

The melanin produced during an inflammatory event can enter the dermis where it is engulfed by macrophages, producing “melanophages”. These cells are often retained in the upper dermis for prolonged periods, as removal of dermal melanin apparently is a very slow process [17]. The major source of color in human skin derives from the presence within the epidermis of specialized melanin the melanosomes. Melanosomes synthesized by melanocytes are acquired by keratinocytes and transported within them to the epidermal surface [16]. The base produced no effects on skin melanin content with respect to time and significant effects with respect to volunteers. The formulation produced significant effects on skin melanin with respect to volunteers and time. The decrease in skin melanin content after application of formulation may be attributed to the antioxidant activity of pomegranate extract which is rich in ellagic acid, punicalgin and vitamins C [7]. Ellagic acid causes inhibition of tyrosinase and melanin synthesis thus inhibiting melanogenesis [19]. Vitamin C promotes collagen growth to maintain and improve tone, firmness, elasticity and flexibility [9].

Inflammation may result in hyperpigmentation through several mechanisms. Among them is direct stimulation of melanocytes by inflammatory

mediators such as Interleukin-1-alpha, endothelin-1, and stem cell factor. Reactive oxygen species, such as superoxide and nitric oxide, generated in damaged skin (for example, from UV exposure) or released as by-products from inflammatory cells are also known stimulators of Melanocytes [18]. For the safety of cosmetics, the important point is that cosmetics must not cause any contact dermatitis when applied to the skin. 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 [20].

The base produced no effects on skin erythema at different time intervals, whereas application of formulation produced significant ($P<0.05$) effects on skin erythema at time and volunteer level. With the help of paired sample t-test it was evident that there was no significant variation in irritation with respect to the base and formulation throughout the study period. As pomegranate extract is rich in ellagic acid, vitamin C and contain potent antioxidant which have anti-inflammatory effects on skin and therefore reduced erythema [17]. Pomegranate has antioxidant, anti-inflammatory and anti carcinogenic potential [6, 21].

The moisturizing treatment 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. The base affected no change in moisture content with respect to the time and significant change ($P<0.05$) with respect to volunteers throughout the study period of 60 days whereas the formulation showed a significant ($P<0.05$) variation throughout the study period of 60 days with respect to time and volunteers. An insignificant change in moisture content was observed throughout study period. Also, insignificant differences in the moisture values were observed after application of the base and the formulation throughout the study period, except after 4th, 6th and 8th week. The significant increase in moisture retaining capacity on skin after application of formulation may be because of vitamin C because collagen synthesis increases and hydration level improves [9].

Face is covered by lipid film derived from sebum and epidermal lipid. Of the two, sebum, which is secreted by the sebaceous glands, is the major component of the lipid film. Sebum secretions vary individually according to age, sex, inherited traits, and topographical variations of the skin [22]. Sebum, the product of sebaceous glands, is a complex of various lipids that are thought to act as an epidermal and/or follicular lubricant [23]. The probe is then placed into the device which radiates a light beam onto the film. A metal mirror behind the film reflects the beam back again through the film and then into an instrument called a photomultiplier, which measures the amount of light in the beam. The more sebum on the skin, the more transparent is the film and greater is the amount of light reflected.

In this study, the effects of the base and formulation on the sebum contents of human cheeks were investigated. Sebum was measured every week in all the individuals. The base increased the sebum contents throughout the study period of 60 days. However, there was an insignificant effect of the base and the formulation on skin sebum throughout the study period. The increase in sebum content after application of the base and the formulation may be attributed to the oily nature of w/o emulsion having a thick viscous oily liquid, i.e., the paraffin oil [24].

3.3. Trans Epidermal Water Loss (TEWL)

Skin barrier capacity is measured by an increased transepidermal water loss. TEWL measures the difference in vapor pressure at two points situated on a line perpendicular to the skin surface inside a tunnel pressed toward the skin. In the absence of skin irritation, TEWL may be considered an indirect measure of stratum corneum water permeability [19].

The changes in TEWL produced by the base and formulation were insignificant. So due to moisture retaining properties; the formulation and the base enhanced the stratum corneum ability to attract, hold and redistribute water thus reducing the TEWL. Of the oils, a mineral oil is frequently used ingredient but can reduce trans epidermal water loss only by about 30% [20].

3.4. Panel Test

A questionnaire containing seven questions was prepared and the two copies of this form were given to each volunteer for sensory evaluation of the two creams. Average points were calculated from the points assigned by each volunteer for each question for both of the creams. Average points for the first question, i.e. ease of application of creams were found to be 4.02 and 4.18 for the base and formulation, respectively. This indicated that the base and formulation can be easily applied on the skin. Average points regarding spreadability were 4.36 for the base and 4.37 for formulation which meant that the formulation spread on skin better than the base. Average points for feel on application were 3.84 for the base and 3.68 for formulation. This indicated that the base and formulation were felt well on the skin. Average points for the sense in long-term application of creams were 4.08 and 4.00 for the base and formulation respectively. This showed that formulation and the base produced pleasant feeling on application to skin. There was no irritation on the skin in both cases i.e. the base and formulation, as these were assigned 0.00 point for irritation by all the volunteers. Shine on skin was 4.00 for the base and 4.04 for formulation. This was expected since the base and formulation contained same quantity of paraffin oil. Similarly, the formulation led to more softness of the skin than the base as the average point was 4.41 for the base and 4.43 for formulation.

The paired sample t-test results revealed no difference between the average points of sensitivity for the base and the formulation. Thus, both of the creams behaved similarly from the sensory point of view.

4. CONCLUSIONS

From this investigations we concluded that a stable W/O emulsion containing extract of pomegranate can be formulated. Significant decreases in skin melanin content of the volunteers after application of the formulation indicated that the formulation possessed skin depigmenting effects. The skin erythema content of the skin decreased after application of the formulation, and did not cause

skin irritation throughout the study period. The formulation produced a pronounced increase in moisture content of the skin at the end of study period. Significant decrease in TEWL indicated that the formulation had anti-wrinkle effects. Thus, this investigation revealed a promising future use of pomegranate extract in skincare formulations without any adverse dermal toxic effects, cosmetics and/or pharmaceutical preparations.

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