Most of cosmetic products are emulsions (1) that function as drug delivery vehicle to the skin and are classified according to different body parts to be applied i.e., skin, teeth, hair and mouth. Emulsions are heterogeneous systems and consist of mixture of two immiscible liquids i.e., aqueous phase and oil phase and system is stabilized by the presence of third substance known as emulsifying agent or emulsifier (2). Main ingredients of these formulations are emulsifier, viscosity modifiers and some active ingredients having auspicious skin care properties. Peculiarly the use of botanical extracts with antioxidant activity provides most valuable cosmetic products. There is exorbitant curiosity about the use of herbal extracts containing phytochemicals with high antioxidant potential and play an important role in eviction of various degenerative diseases and production of modern phytocosmetic preparations (3). Most important bioactive phytoconstituents of plants include polyphenols, flavonoids, alkaloids, saponins, tannins and essential oils (4). The topically applied cosmetic preparations are more beneficial in respect to solubility, spreadability, enhanced bioavailability (avoid first pass effect) and topical delivery of hydrophilic and hydrophobic ingredients (5).

Skin is the largest and outermost organ of the body also known as cutaneous membrane or the integumentry system (6). The skin constitutes 7% of total body weight and about 0.02” to 0.16” in thickness in adults (7). Human skin acts as a frontier between interior and exterior of the body and performs various important functions. It regulates the transport of water and other substances with environment and is responsible for defensive mechanisms such as protection from environmental hazards i.e., chemical, physical, biological and microbial. It also protects the body from lethal effects of...
solar radiations and reduces the free radical production and overall oxidative stress in the body. It is also responsible for thermoregulation during extreme temperature conditions (8). Skin consists of many layers among which stratum corneum (SC) is the super facial, tough and water repellant layer and act as main barrier by regulating the selective permeability of substances in and out of the skin (9, 10). Similarly, water loss can also be diminished due to the presence of glycolipids in epidermal layer of the skin. Dermis contains collagen and elastic fibers which together constitute a protein called elastin and regulate the elasticity of skin (11). Dermis also contains glycoprotein and holds large quantity of water (12). Morphological changes that cause disturbance in this barrier result in enhanced transepidermal water loss (TEWL), which leads towards the degeneration of collagen, loss of elasticity, high roughness, atopic dermatitis and states the aging process (13, 14). Use of moisturizing agents on daily basis is helpful in maintaining the skin barrier function and prevents water loss, skin dryness or roughness and atopic dermatitis (15).

Due to these grounds there is more attention towards investigation of the plants and phytoconstituents having moisturizing and anti-aging properties. Phenolics are reported antioxidant compounds due to excellent free radical scavenging activity and provide emollient, moisturizing and anti-aging effects in various skin care products (16). Ananas comosus L. is a tropical fruit belonging to the family Bromeliaceae abundant in phenolics i.e., caffeic acid, myrecetin, anthocyanins, catechin, flavones and isoflavones. It has reported free radical scavenging activity and interferes with propagation and formation of free radicals (17). Thus, it should be considered valuable for maintaining proper health conditions and provides the basis for the selection of this fruit for skin care formulations. Non-invasive measurements of skin hydration and TEWL values became possible due to advancement in biometrological techniques. The present study was aimed to formulate skin care cream (w/o emulsion) entrapped with *Ananas comosus* extract and assessed for stability testing and evaluated non-invasively for changes in skin barrier functions for a period of 90 days.

**EXPERIMENTAL**

**Plant identification**

Identification of *Ananas comosus* fruit was conducted from Cholistan Institute of Desert Study (CIDS), The Islamia University of Bahawalpur. The voucher no. is 3521/CIDS/IUB, and specimen was deposited in Pharmacognosy lab, Pharmacy Department, The Islamia University of Bahawalpur, Pakistan.

**Material**

Ananas comosus was purchased from model Bazaar, Bahawalpur. Paraffin oil (h: 110-230 mPa·s, at 20°C) was obtained from Merck (Germany), Polysiloxane polyalkyl polyether copolymer (Abil-EM90 with HLB 5) was purchased from Franken Chemicals (Germany), Distilled water was prepared by using distillation plant (Irmeco-GmbH, Germany) and *Ananas comosus* extract was prepared in cosmetic laboratory of Pharmacy Department, The Islamia University of Bahawalpur, Pakistan.

**Apparatus**

The following apparatus was used to perform the experiments: electrical balance (Precisa BJ-210, Switzerland), rotary evaporator (Eyela, Co. Ltd., Japan), water bath (HH.S21 4, China), centrifuge machine (Hettich EBA 20, Germany), hot incubator (Sanyo MIR-162, Japan), cold incubator (Sanyo MIR-153, Japan), pH-meter (WTW pH-197i, Germany) and refrigerator (Orient, Pakistan).

**Skin hydration and transepidermal water loss (TEWL) measurements**

In this study the measurement of skin hydration levels were executed using non-invasive biometrological probes. The Corneometer® (Courage & Khazaka, Germany) was used to measure water contents of stratum corneum by electrical capacitance probe and expressed the values in arbitrary units (a.u.) ranging from 0-120 a.u. so called corneometric indexes (18). Probe was applied to skin with standard force of 3.5 N and measured the hydration levels of superficial epidermis down to the depth of about 0.1 mm and results were shown digitally in arbitrary units within 3 s. The measurement of TEWL was conducted by a Tewameter® MPA 580 (Courage & Khazaka, Electronics GmbH, Cologne, Germany) which is calibrated according to manufacturer’s guidelines and based on the principles of diffusion in an open chamber and measures in g/m²/h. The surface evaluation of living skin (SELS) were evaluated by image analysis; using Visioscan, VC 98/software SELS 2000 (Courage & Khazaka GmbH) in a 15317 mm area.

**Preparation of Ananas comosus extract**

The peel of pineapple fruit was trimmed and fruit was spliced into small pieces. A paste was pre-
pared by homogenization in a grinder for 5 min. Hundred grams of paste material was extracted with 300 mL of methanol with magnetic stirrer at 30°C for 2 h. The extract was separated from residue by filtering through Whatman No. 41 filter paper. Extract was concentrated and solvent was removed under vacuum using rotary evaporator (EYELA, CA-1111, Rikakikai Company Ltd., Tokyo, Japan) at 40 ± 5°C. The dried fruit extract was stored at 2-8°C in a refrigerator, until used for further investigation.

**Free radical scavenging activity of extract**

The DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical method was used to assess the antioxidant activity of the extract (19). Ten µL of extract of fruit was added in a 96-well plate and made up the total volume up to 100 µL with DPPH solution. Similarly added was 10 µL of ascorbic acid as standard and made up the volume up to 100 µL with DPPH solution. Then, both mixtures were incubated at 37°C for a period of 30 min and the absorbance was measured at 517 nm. Experiment was performed in triplicate and results were taken as the mean.

\[
\% \text{ Inhibition} = \left( \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \right) \times 100
\]

**Formulation development**

In present study, creams (w/o emulsion) were prepared by addition of aqueous phase into oil phase by continuous stirring (20). For development of active cream, oily phase consisted of liquid paraffin (14%) and emulsifying agent Abil®EM 90 (3.5%) was heated up to 75 ± 1°C. At the same time, aqueous phase consisting of deionized water (79.50%) was also heated at the same temperature in water bath (HH.S21 4, China). Then, *Ananas comosus* extract was dissolved in aqueous phase. After that, aqueous phase was poured slowly into oily phase with continuous stirring at 2000 rpm for 15 min using digital homogenizer (IKA Werke, Germany) until complete aqueous phase was added. Then, stirring speed was reduced up to 1500 rpm for homogenization up to 10 min. After that, speed was further reduced to 500 rpm until formulation cooled to room temperature (21). The same procedure was adopted for preparation of placebo (base) but without addition of fruit extract. Compositions of active cream and placebo are shown in Table 1.

**Subjects**

In this study, 11 healthy male volunteers (mean age 27 ± 0.45 years) were selected. All volunteers were evaluated by a cosmetic expert for any kind of skin disease, particularly on areas specified for application of creams. Individuals with found sensitivity to cream ingredients, having extraordinary hairs on cheeks and did not follow the study protocol were excluded from the study. Individuals were instructed to avoid the use of any other moisturizing creams on application area and only use normal cleansing products 15 days prior to study and during entire period of 12 weeks. Moreover, they were also instructed to continue their routine diets during study period in order to nullify the effects of such changes on the results of study.

**Study design**

Physical stability of creams were assessed by placing various samples (50 g) of base and formulations in glass beaker and covered with aluminum foil at 8 ± 0.1°C (in refrigerator), 25 ± 0.1°C, 40 ± 0.1°C, 40 ± 0.1°C with 75% RH (relative humidity) and 50 ± 0.1°C (in incubator). Different physicochemical parameters i.e., color; centrifugation, liquefaction, pH and viscosity were assessed during different time intervals for three months study period in order to assure desired stability of creams.

In *vivo* measurements for this placebo controlled, split face, single-blinded study was executed during winter (September to December) in draught free room with controlled conditions of temperature (22-25°C) and relative humidity (55-60%) for a period of 12 weeks. Manufacturer’s instructions were followed by authors to execute the instrumental measurements by a single cosmetic expert to avoid person to person variation. Then, every individual was provided with two vessels containing 40 g of active cream and placebo, marked “right” and “left”, respectively, to be applied on respective cheeks daily at bed time, while area around the eyes

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Paraffin oil</th>
<th>Abil EM 90</th>
<th>Fruit extract</th>
<th>Distilled water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>14%</td>
<td>3.5%</td>
<td>-</td>
<td>q.s.100%</td>
</tr>
<tr>
<td>Active</td>
<td>14%</td>
<td>3.5%</td>
<td>2%</td>
<td>q.s.100%</td>
</tr>
</tbody>
</table>
were omitted. The volunteers were instructed about the proper use of creams and reminded daily to assure 100% compliance. Before execution of measurements, the volunteers had to stay in the laboratory for at least 15 min in order to adjust the skin according to environmental conditions (22 ± 2°C and 60 ± 5% relative humidity) in compliance with the procedures set for these measurements.

**Ethical considerations**

For the execution of the study the acceptance was taken by Institutional Ethical Committee (IEC), Faculty of Pharmacy and Alternative Medicine. Study was also approved by Board of the Advanced Study and Research (BASAR), The Islamia University Bahawalpur, Pakistan (No. 974/AS&RB). The study was conducted with notification of Helsinki and was regulated with good clinical practice guidelines. Written informed consents were signed by all volunteers prior to the study and they were informed about possible adverse effects and study protocols. They had the right to discontinue the study without notify about such reasons.

**Statistical analysis**

The data for measured parameters (pH, skin hydration and TEWL) were analyzed statistically using SPSS v.17 software. Two-way ANOVA and paired t-test was used to analyze the variation between two preparations, while \( p \leq 0.05 \) values were considered statistically significant. The rheological parameters were estimated using power law mathematical model. Difference from baseline values at various time intervals was indicated by percentage changes by the following formula:

\[
\% \text{ change} = \left( \frac{D_x - D_0}{D_0} \right) \times 100
\]

where \( D_x \) indicate value obtained at different time intervals and \( D_0 \) indicate the baseline value.

### Table 2. Stability study profile of both active (A) and placebo (P) creams with time.

<table>
<thead>
<tr>
<th>Physical parameters</th>
<th>Fresh</th>
<th>12 h</th>
<th>24 h</th>
<th>48 h</th>
<th>7 d</th>
<th>21 d</th>
<th>28 d</th>
<th>60 d</th>
<th>90 d</th>
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<tbody>
<tr>
<td>P</td>
<td>A</td>
<td>W</td>
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<td>W</td>
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<td>A</td>
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<td>A</td>
<td>P</td>
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</tbody>
</table>

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<th>12 h</th>
<th>24 h</th>
<th>48 h</th>
<th>7 d</th>
<th>21 d</th>
<th>28 d</th>
<th>60 d</th>
<th>90 d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color</td>
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<td></td>
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<td></td>
<td></td>
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<tr>
<td>A</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>B</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>C</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>D</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
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<td>S</td>
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<td>S</td>
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<tr>
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<td>S</td>
<td>S</td>
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<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
</tbody>
</table>

Table 3. pH value (mean ± standard error of mean) at different storage conditions.

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Fresh</th>
<th>8°C</th>
<th>25°C</th>
<th>40°C</th>
<th>40°C ± 75% RH</th>
<th>50°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>6.42 ± 0.08</td>
<td>6.321 ± 0.11</td>
<td>6.092 ± 1.02</td>
<td>5.967 ± 0.35</td>
<td>5.912 ± 0.46</td>
<td>4.819 ± 1.09</td>
</tr>
<tr>
<td>Active</td>
<td>6.13 ± 0.10</td>
<td>6.103 ± 0.12</td>
<td>5.847 ± 0.87</td>
<td>5.429 ± 0.12</td>
<td>5.202 ± 1.34</td>
<td>5.026 ± 0.28</td>
</tr>
</tbody>
</table>

RH = relative humidity.
Experimental error is expressed in the table as the standard error of measurement (SEM).

RESULTS

Antioxidant activity

Antioxidant or free radical scavenging activity of Ananas comosus extract was measured using DPPH free radical method and was found to be 92% compared to the standard antioxidant i.e., ascorbic acid.

Stability assessment of creams

Accelerated conditions of temperature and centrifugation test are important parameters to access the physical stability of creams (22). Stability of creams (active and placebo) were evaluated at various storage temperatures i.e., 8, 25, 40°C, 40°C ± 75% RH (relative humidity) and 50°C for a period 12 weeks. Results indicated that no changes in color and liquefaction were found in active and placebo creams at all storage temperatures (Table 2). Similarly, no phase separation was observed in active cream and placebo control kept at various temperatures with centrifugation test performed immediately after preparation and at various time intervals (24 h and on days 7, 15, 30, 45, 60, 75 and 90) (Table 2). It was obvious from the literature that the stability of emulsion under stress conditions could be enhanced by maintaining proper homogenization speed during preparation of emulsion (23). Electrical conductivity test was found negative in all the samples of active and placebo creams indicating the type of formed emulsion i.e., water in oil (w/o). This was because oil, being the continuous phase, not contributes to passage of electrical current (24).

pH analysis

The pH analysis of creams is an important parameter regarding stability and efficacy (25). The normal range of skin pH is 4.5-6, so formulations intended to be applied to the skin should have pH closer to this range (26). pH analysis were performed of freshly prepared creams and after (24, 48, 72 h and on days 7, 15, 21, 28, 45, 60 and 90) and average change in pH values are reported in Table 3. It was evident from results that no significant variation was observed in pH values throughout the study period.

Rheological assessment

Rheological analysis of creams is essential to access the optimum stability as well as the changes produced with aging, stress and temperature. It also acts as a preliminary tool for the imminent failure of the product during storage (27). In present study, rheological assessment of placebo control and active cream was performed at 25°C using Brookfield rotational rheometer (Model DV.III; Brookfield Engineering Laboratories, USA) at 100 to 200 rpm
speed (with 30 increments) using spindle CP 41. Data were analyzed with Rheocalc software version (2.6) using power law mathematical model:

$$\tau = K \gamma^n$$

where $\tau$ is shear stress, $\gamma$ is shear rate, $K$ is consistency index, and $n$ is flow behavior index.

It was evident from the results that viscosities of freshly prepared active cream and placebo control were 304.09 cP and 257.50 cP, respectively. Viscosities of both creams i.e., placebo and active, decreased gradually with increased shear rate, which indicated shear thinning or pseudoplastic behavior of all samples (Table 4 and Fig. 1). The value of flow index was found to be $\leq 1$ also indicating pseudoplastic flow of prepared creams, which is satisfactory rheological parameter due to formation of coherent film which covers skin surface upon application (28).

### Evaluation of epidermal hydration level

Percentage changes in epidermal hydration level following long term application of active cream and placebo control along with statistical interpretation are given in Table 5 and Figure 2. It was evident from the results that placebo side of face showed low epidermal hydration levels at baseline i.e., 1.96%, but increased slightly after 15 days of application (8.13%), and remained consistent till 90 days i.e., 10.23%. Contrary to the placebo, side of epidermal hydration levels were gradually enhanced at active side of face i.e., 2.30% at baseline and reaching a maximum value of 56.74% at the end of the study period. It was evident from statistical interpretation using ANOVA that active side of face showed significant improvements ($p \leq 0.05$) in epidermal hydration levels, while on placebo side insignificant changes in epidermal hydration levels were observed.

![Figure 2. Percentage of change in epidermal hydration level after application of placebo and active creams](image-url)

Table 4. Results of rheological analysis followed for 90 days.

<table>
<thead>
<tr>
<th>Model</th>
<th>Rheological parameter</th>
<th>Fresh</th>
<th>8°C</th>
<th>25°C</th>
<th>40°C</th>
<th>40°C RH</th>
<th>50°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>P</td>
<td>A</td>
<td>P</td>
<td>A</td>
<td>P</td>
<td>A</td>
</tr>
<tr>
<td>Consistency</td>
<td>index (cP)</td>
<td>273.3</td>
<td>260.1</td>
<td>188.3</td>
<td>236.2</td>
<td>159.4</td>
<td>207.7</td>
</tr>
<tr>
<td>Power Law</td>
<td></td>
<td>0.57</td>
<td>0.49</td>
<td>0.62</td>
<td>0.56</td>
<td>0.44</td>
<td>0.39</td>
</tr>
<tr>
<td>Flow index</td>
<td></td>
<td>96.3</td>
<td>96.9</td>
<td>96.6</td>
<td>99.5</td>
<td>99.9</td>
<td>99.4</td>
</tr>
<tr>
<td>Apparent viscosity (cP*)</td>
<td></td>
<td>304.1</td>
<td>257.5</td>
<td>298.1</td>
<td>248.5</td>
<td>293.4</td>
<td>241.6</td>
</tr>
</tbody>
</table>

P = placebo; A = active cream; cP* = mean apparent viscosity at 100–200 rpm, RH = 75% relative humidity.
were observed with respect to time (p value equaled 0.011 and 0.192, respectively). With the help of paired sample t-test it was found that treatment with active cream was superior to placebo control as it significantly increased the epidermal hydration levels throughout the study period.

**Evaluation of transepidermal water loss (TEWL)**

The change in TEWL values after application of active cream and placebo control are given in Table 5 and Figure 3. It was evident from the results that placebo side of face exhibited initial increase in TEWL i.e., 3.12% at baseline and after that a delicate decline in TEWL values was observed i.e., -1.01% after 15 days, -1.96% after 30 days, -3.14% after 45 days, -7.01% after 60 days, and -8.98% after 90 days. In contrast to the placebo side of face, marvelous depreciation in TEWL was observed on active side of face. Reduction in TEWL values occurred gradually in the following order; -20.08%, -31.40%, -40.09%, -57.13%, -66.35%, and -73.19% after 15, 30, 45, 60, 75, and 90 days, respectively, compared to the baseline values i.e., 3.97%. With statistical interpretation using ANOVA it was demonstrated that active cream shows significant (p ≤ 0.05) decline in TEWL while insignificant effects were observed with placebo control at different time intervals (p value equals 0.309 and 0.002, respectively). By applying paired sample t-test a significant (p ≤ 0.05) difference in TEWL was observed between active and placebo control throughout 12 week study period.

### Table 5. Percentage changes in measured skin epidermal function after 90 days topical application of active cream and placebo control (mean ± standard error of mean) with time.

<table>
<thead>
<tr>
<th>Trans epidermal water loss (TEWL)</th>
<th>Formulations</th>
<th>Baseline</th>
<th>2nd Week</th>
<th>4th Week</th>
<th>6th Week</th>
<th>8th Week</th>
<th>10th Week</th>
<th>12th Week</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>3.12 ± 1.02</td>
<td>-1.01 ± 0.34</td>
<td>-1.96 ± 1.04</td>
<td>-3.14 ± 0.02</td>
<td>-7.01 ± 1.25</td>
<td>-8.19 ± 0.61</td>
<td>-8.98 ± 0.76</td>
<td>0.309**</td>
<td></td>
</tr>
<tr>
<td>Active</td>
<td>3.97 ± 1.02</td>
<td>-20.08 ± 0.34</td>
<td>-31.40 ± 1.40</td>
<td>-40.09 ± 1.21</td>
<td>-57.13 ± 0.72</td>
<td>-66.35 ± 0.91</td>
<td>-73.19 ± 1.20</td>
<td>0.002*</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Moisture contents (MC)</th>
<th>Formulations</th>
<th>Baseline</th>
<th>2nd Week</th>
<th>4th Week</th>
<th>6th Week</th>
<th>8th Week</th>
<th>10th Week</th>
<th>12th Week</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>1.96 ± 0.08</td>
<td>8.13 ± 0.08</td>
<td>9.06 ± 1.91</td>
<td>8.47 ± 1.27</td>
<td>8.21 ± 1.89</td>
<td>9.99 ± 0.21</td>
<td>10.23 ± 1.50</td>
<td>0.192**</td>
<td></td>
</tr>
<tr>
<td>Active</td>
<td>2.30 ± 1.20</td>
<td>6.13 ± 1.39</td>
<td>17.14 ± 0.73</td>
<td>22.89 ± 0.44</td>
<td>30.15 ± 1.54</td>
<td>45.31 ± 0.53</td>
<td>56.74 ± 0.92</td>
<td>0.011*</td>
<td></td>
</tr>
</tbody>
</table>

*p ≤ 0.05 (significant), NS = non significant change

Figure 3. Percentage of change in skin transepidermal water loss (TEWL) after application of placebo and active creams
Surface evaluation of living skin (SELS)

The surface evaluation of living skin (SELS) i.e., roughness (SEr), scaliness (SEsc), smoothness (SEsm) and wrinkles (SEw) were evaluated by image analysis taken under certain illumination; using Visioscan, VC 98/software SELS 2000 (Courage & Khazaka GmbH). The values (the mean ± SEM) of different SELS parameters SEr, SEsc, SEsm and SEw measured before application of creams (baseline readings) and then at 4th, 8th and 12th week of study period are given in Table 6. It was obvious from the SELS evaluation that SEr, SEsc, SEsm and SEw were declined significantly (p ≤ 0.05) with active cream while insignificant results were produced by placebo control when ANOVA was applied. The active cream indicates a significant effect when paired sample t-test was applied throughout the study period.

DISCUSSION

Normal homeostasis is maintained against ROS or free radicals through a controlled mechanism due to existence and activation of endogenous antioxidants. However, when there is an insufficiency or imbalance of endogenous antioxidants to combat the ROS or free radicals, then exogenous antioxidants may help to rehabilitate this balance. Antioxidants defend the body against ROS by directly scavenging the free radicals, terminate their propagation during lipid peroxidation, block their access to the biological targets thus impeding the overall oxidative stress and slowing the process of aging. Ananas comosus extract serves binary function in this regard i.e., firstly interferes with propagation and secondly intercepts the formation of free radicals (17).

In order to access the in vivo skin barrier function, the most important method is to measure the transepidermal water loss (TEWL), as it demonstrates the outward loss of water through the skin and reflects the skin water contents (29). Some of the important variables that affects the TEWL measurements are ambient air temperature and humidity, site of measurement, inter and intra-individual differences and instrumental related variables (30). Moreover, an inverse relationship exists between TEWL and epidermal hydration level i.e., elevated TEWL values are an indication of impaired water barrier and it results in low hydration or moisture of epidermal layer of skin as manifested by various skin diseases (31).

Results of our study are analogous with the above mentioned hypothesis. As active side of face showed gradual improvement in epidermal hydration levels of skin from baseline value and reached a maximum value of 56.74% at 90th day of study, TEWL values on active side also decreased gradually throughout the study period and reached -73.19% at 90th day of study. On the other hand, statistical interpretations indicate that significant (p ≤ 0.05) improvements in epidermal hydration levels appear on active side of face due to hyper hydration and reduction in water loss compared to placebo side of face which shows insignificant results on epidermal hydration levels. Thus, creams loaded with Ananas comosus extract demonstrate superior outcomes on skin barrier function compared to placebo control.

Exposure to UV radiations could produce serious damage to skin barrier and results in loss of water from skin. Water binding affinity of stratum corneum diminishes this water loss known as stratum corneum moisture contents, but excessive loss of water from stratum corneum (= 10%) due to disruption of skin barrier results in degeneration of collagen, skin dryness and loss of flexibility (32). Phenolic contents i.e., quercetin and catechin have reported moisturizing and anti-solar properties so,
topical application of *Ananas comosus* cream drenched with phenolic contents protects the skin against UV induced destruction of skin barrier and improves the hydration of the stratum corneum (33, 34). Similarly, flavonoids as an important constituent of *Ananas comosus* extract also improved skin moisture contents due to swelling of corneocytes at skin surface (35). The results of our study are in close agreement with the literature findings that plants rich in polyphenols and flavonoids provide protection against UV induced photooxidation of proteins and lipids, oxidative stress and prevent the inflammation of skin by improving collagen efficiency thus improving the skin barrier function (36).

It has been reported that *Ananas comosus* extract contains certain vitamins i.e., A, B and C (17). It was evident from the literature that vitamin C (ascorbic acid) was found in concentration of 0.4 to 1 mg/100 g of wet tissue weight in structural organization of lipid barrier of stratum corneum and normalizes the epidermal lipid profiles in redeveloped epidermis (37). In addition, vitamin A and its derivative β-carotene (provitamin A) demonstrated photoprotective effects due to increased protein and collagen synthesis and eventually enhanced epidermal thickness so were used as an admirable ingredient in cosmetic preparations (38). Similarly, vitamin B belongs to a group of hydrophilic nutrients and acts as humectants i.e., retards the water loss from stratum corneum, softens the skin, so was used as an emollient and moisturizing agent in cosmetic products (39). In the present study, the possibility of increased epidermal moisture contents by application of active cream may be due to existence of above mentioned vitamins in *Ananas comosus* extract. Including all of these, in prepared topical creams (w/o) oil becomes the continuous phase which acts as an occlusive dressing on skin surface, reduces TEWL and enhances epidermal hydration level, whereas lipids gain access through the defiled barrier and also enhance barrier recovery. Higher epidermal hydration levels also corresponds with decreased skin roughness (SEr) and scaliness (SEsc) (40). Due to the presence of polyphenols and flavonoids, *Ananas comosus* active cream improves the elasticity of connective tissues hence reduces the appearance of skin wrinkles (SEw) and delays the skin aging (41).

Recent studies revealed that impaired skin barrier causes the development of atopic dermatitis (AD) which is multifactorial inflammatory skin disease. The severity of AD increased with increased deficiency in barrier function of the skin (42). It was obvious from the results that skin barrier function was improved with application of active cream, so in order to explore the actual potential of this fruit, future investigations are required in patients with compromised skin barrier function, especially against AD.

**CONCLUSION**

As a conclusion of this study, topical creams (w/o emulsion) containing extract of *Ananas comosus* and placebo were formulated. The creams acquired good cosmetic appeal and physical stability with complete absence of color change and phase separation. It was also concluded that active cream reduces TEWL and SELS parameters and improved epidermal hydration levels, thus may be used as an ingredient in moisturizing cosmetic preparation to treat dry skin conditions, halting the degeneration of collagen, loss of skin elasticity and appearance of wrinkles and depreciating visible signs of aging. In addition, active cream also improved the skin barrier function and should be fruitful in the management of atopic dermatitis. Since promising results were obtained with quantitative measurements of TEWL and moisture contents following long term application of *Ananas comosus* L. in healthy volunteers so, future investigations are mandatory to clinically explore these extract in patients with compromised skin barrier particularly against AD.

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