APPLICATIONS OF A STABLE GREEN TEA EXTRACT CREAM ON HUMAN CHEEKS

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ABSTRACT

The study was aimed to formulate a stable dermatological formulation of the green tea extract and to explore its effects on skin hydration and trans epidermal water loss (TEWL) of the skin. Any effects produced were measured with Corneometer and Tewameter (Non-invasive probes).

For this study design two formulations were developed. Formulation one the Base formulation in which no green tea extract was incorporated while second one was the Active formulation in which preconcentrated ethanolic green tea extract was incorporated into a W/O emulsion using ABIL®EM90 as an emulsifier. Different formulations so formed were tested for stability studies for a period of 28 days using various conditions of temperature and humidity i.e. 8°C, 25°C, 40°C & 40°C+75% RH (Relative Humidity). A stable formulation with 3% green tea extract was selected for the desired study and was applied to the cheeks of human volunteers (n=10) for a study period of 8 weeks. Results of each individual for skin hydration and TEWL were analyzed by Corneometer and Tewameter. By using ANOVA and Paired t-test as statistical evaluation techniques, results of both the Base formulation and Active formulation were compared for produced effects. Statistically significant results were found for skin hydration and TEWL when the Active formulation was compared with the Base formulation. Hence desired formulation can be helpful in rejuvenating the skin.

Key words: Camellia sinensis, Skin Moisture, TEWL

1. INTRODUCTION

Skin being the largest organ of our body is supplied frequently with blood circulation and is considered excellent model for topical delivery. For the dermatological formulations emulsions have been used since long time applied as topical emulsions or as emulsions with defined percutaneous absorption. Main advantage of emulsions is there widespread acceptability by the user [1]. Among the various types of emulsions W/O type emulsion are frequently used because of their ease of spreadibility and furthermore these emulsions restrict evaporation of water from skin surface, thus hindering dehydration of the skin by their emollient action [2].

Worldwide tea is the most widely consumed beverages due to its unique aroma, taste and certain health promotion properties. It is reported that tea contains almost 4000, bioactive compounds among one third part is contributed by polyphenols. These polyphenols are bonded benzene rings with multiple hydroxyl groups. Usually Polyphenols are either flavonoids or non-flavonoids but most of the chimicals in tea are flavonoids [3]. Catechins (polyphenols) are main active constituents found in tea [4]. Environmental oxidative damages are among major risk factor to all living organisms. Increased production of free radicals gives rise to these damages. Sources for these radicals are either endogenous such as inflammation exogenous source such as pollution, radiation and cigarette smoking. Radicals with unpaired electron tend to react with other molecules to trap electron away from them thus starting a chain reaction. Almost all types of organ and tissues are subjected to radical damages but intensity of damage may vary. Plant secondary metabolites gained lot of attention of researchers to combat these degenerative diseases [5]. Tea preparations have shown to traping activity against various reactive oxygen species (ROS) such as singlet oxygen, hydroxyl radical, superoxide radical, nitric oxide, peroxynitrite and nitrogen dioxide and were helpful in reducing damage to proteins, lipid membranes and nucleic acid in cell-free systems [6].

Remarkable health properties of tea were reported in many epidemiological studies using animal models [7]. The problem with green tea polyphenols was their strong affinities for many proteins like milk, casein, gelatin and saliva when administered orally. Interfering of polyphenols with digestive enzymes was another problem that results in reduced lipid, starch and proteins digestibility. Moreover they have shown to interfere with zinc, iron and sodium as well [3]. Therefore a desired dermatological formulation was of esteemed value using green tea extract. Green tea extracts are utilized either in liquids (infusions) form or as dry extracts for further purification of the extract for its active constituents [8].

2. EXPERIMENTAL

2.1. Materials

Camellia sinensis leaves were purchased from Pak Sea Buckthorn International Skardu, Pakistan and ABIL®EM90 was purchased from Franken Chemical (Germany). Paraffin oil was purchased from Merk KGaA Darmstadt (Germany). Ethanol was taken from BDH England.

The identification of *Camellia sinensis* (Family: Theaceae) was confirmed at Cholistan Institute of Desert Studies (CIDS), The Islamia University of Bahawalpur, Pakistan.

2.2. Apparatus

Centrifuge Machine	Hettich EBA 20, Germany
Cold Incubator	Sanyo MIR-153, Japan
Conductivity-Meter	WTW COND-197i, Germany
Corneometer MPA 5	Courage + Khazaka, Germany
TEWAmeter 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

2.3. Methods

2.3.1. Preparation of Extract

Camellia sinensis leaves extract was prepared by macerating 200g finally powdered *Camellia sinensis* leaves with one liter of analytical grade ethanol in a glass beaker. Glass beaker was sealed with aluminum foil and kept at room temperature for seven days. After seven days fresh 500 ml analytical grade ethanol was added and kept it for further one week. Ultimately after two weeks a quantity 500 ml of fresh menstrum was added to further macerate it for one more week. The beaker was shaken for 10 minutes after every 24 hours. Macerated plant material was finally filtered through several layers of muslin cloth for coarse filtration. The coarse filtrate was finally filtered through Whatman # 01 filter paper.

The filtrate so obtained was evaporated at 40°C under vacuum, using a rotary evaporator. The evaporation process was continued till concentrate reduced to one seventh of the total volume used. The blackish green colored extract so obtained was collected in stoppered glass tubes and stored in freezer at 8°C till its use.

2.3.2. Preparation and application of Creams

For a *Camellia sinensis* (Green tea) extract cream an oily phase that consisted of paraffin oil (16 %) and surfactant ABIL®EM 90 (5 %) was heated up to 75°C±1°C. At the same time, aqueous phase consisting of water (quantity sufficient to make 100 %) was heated to 75°C±1°C and then the green tea extract (3 %) was added in this aqueous phase. After that, aqueous phase was added to the oil phase drop by drop using a stirring speed of 2000 rpm by the mechanical mixer for about 15 minutes until complete aqueous phase was added. Mean while few 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. A similar procedure was followed for developing a control base formulation except for no green tea extract was added in it.

Creams (Formulations) were organoleptically analyzed for color, thickness, feel and look and also observed physically for creaming and phase separation. Both formulation and base were checked for their pH, electrical conductivity and any phase separation using appropriate technology.

For application of creams 10 volunteers were chosen whose ages were in between 25 and 40 years. Only male volunteers were included in this work. Volunteers were examined for any serious skin disease or damage especially on cheeks and forearms. Every volunteer was provided with a volunteer protocol before the study. This protocol stating every volunteer signed the terms and conditions of the testing individually. Volunteers were not informed about the contents of the formulations. Skin tests were performed at 25°C and 40% relative humidity conditions. On the first day, patch test was performed to determine any possible reactions to the formulations, on the forearms of each volunteer. Each volunteer on the second day was provided with two creams. One cream was the Base formulation and other was the Active formulation. Each volunteer applied the creams for 8 weeks of study period. Every individual was instructed to come for measurements on week 1, 2, 3, 4, 6, and 8.

At the end of the study 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.

2.3.4. Mathematical and Statistical Analysis

The percentage changes for the individual values of both the parameters were calculated by the following formula;

Percentage Change = [(A – B) / B]*100

Here;

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

B = Zero hour value of that parameter

The measured values obtained for skin hydration and TEWL 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).

3. STUDY DESIGN

A single-blinded study with placebo control was designed for comparison of two creams i.e. the Base formulation and the Active formulation. Two formulations were named A (active formulation) and B (base). Volunteers (n=10) with ages between 25 and 40 with normal skin were asked to sign a consent form to start a study of 60 days. Then two creams A and B were given to each volunteer with necessary instructions of application. Results were measured with suitable techniques in a controlled room at 21˚C ± 1 and 40˚C ± 2 % relative humidity. All volunteers finished the study and compliance was verified by weighting the sample before and after study. At the 60th day volunteers were asked to fill a form of Panel test for sensory evaluation of the creams.

4. RESULTS AND DISCUSSION

4.1 In-vitro physical evaluation of creams

Stability of both the Base and the Active formulation was evaluated using different conditions of storage i.e. 8˚C, 25˚C and 40˚C + 75% RH and physical characteristics were observed for any change after these conditions. Accelerated temperatures and centrifugation are considered important for long term stability of the emulsions. For example centrifugation at 15,400 g for a 5 hours period would be sufficient to state the emulsion stability for a period of one year [9]. In this study no phase separation in samples kept at different storage conditions was observed for 28 days at different time intervals by performing centrifugation tests both for base and formulation. As elevated temperatures cause change in viscosity, solubility and partitioning of molecules between two phases. But it has been found that lipophilic surfactants are more stable at elevated temperatures [9]. As ABIL®EM90 is lipophilic surfactant [10]. No liquefaction was observed in the formulation throughout the study period of 28 days. However slight liquefaction was observed in base in last week of the 28 days study period.

4.2. In-vitro evaluation of pH of creams

Average changes in pH values of both base and formulation samples kept at different storage conditions up to 28 days have been determined and results are shown in Fig. 1. Normal skin pH ranges from 4-6.5 and varies in different areas of the skin. Skin bears an acid mantle which is fine film on skin surface with slightly acidic pH. This acid layer provides skin protection against microbes and protects from alkaline substances by acting as alkali neutralizing agent [11].

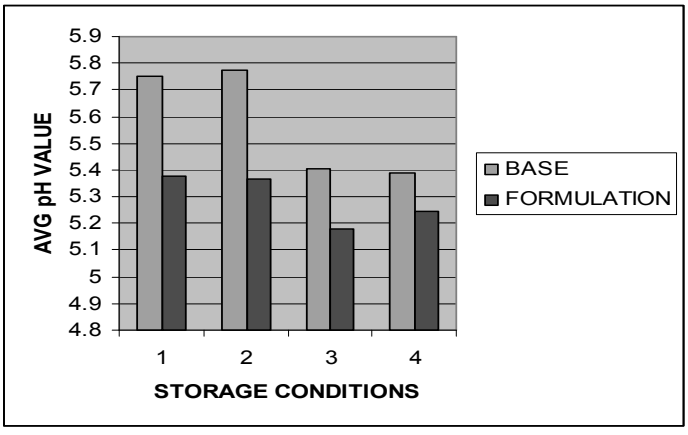

Fig. 1. *pH Values of Base and Formulation Kept at 8°C, 25°C, 40°C and 40°C + 75% RH*

RH = Relative Humidity. 1= 8˚C, 2= 25˚ C, 3= 40˚C and 4= 40˚ C+ 75% RH

In present study pH of the Base formulation was 5.76 while of the Active formulation was 5.67 indicating a good skin pH range. Samples of the base kept at different storage conditions i.e. 8°C, 25°C, 40°C and 40°C + 75% RH showed slight increase in pH in 3rd and 4th week. While formulation samples showed reduction in pH at all time intervals and test conditions. By using two way ANOVA as statistical evaluation technique, pH of base samples found insignificant with respect to time while formulation samples showed significant pH results with respect to time. While LSD test was applied for formulation samples, it was significant from 2nd day to the end of the study. The decrease in pH with formulation sample was due to phenolic acids such as Gallic acid present in the green tea [12].

4.3. In-vivo characterization of creams for skin Hydration and TEWL

4.3.1. Patch Test

A patch test was performed on forearms of the volunteers for 48 hours to check any irritation either in the Base or in the Active formulation. Though a slight increase in erythema level was observed with formulation after 48 hours but statistically these effects were insignificant (Paired sample t-test). It was concluded that both the base and formulation can be applied to the cheeks of volunteers without any risk of skin irritation.

4.3.4. Skin Moisture

A dry flaky skin requires hydration of the stratum corneum. It is usually achieved by the application of moisturizers. Moisturizers reduce evaporation of the water outside the skin, the action so called occlusion. Hydrophobic substances such as lipids are well known for their occlusion potential, thus minimizing the water loss from the skin [13].

In this study the Base formulation improved the moisture contents of the skin with some irregular pattern but formulation showed a rhythmic increase in water contents of the skin and results for the average percent change occurred in the skin moisture content are showed in Fig. 2.

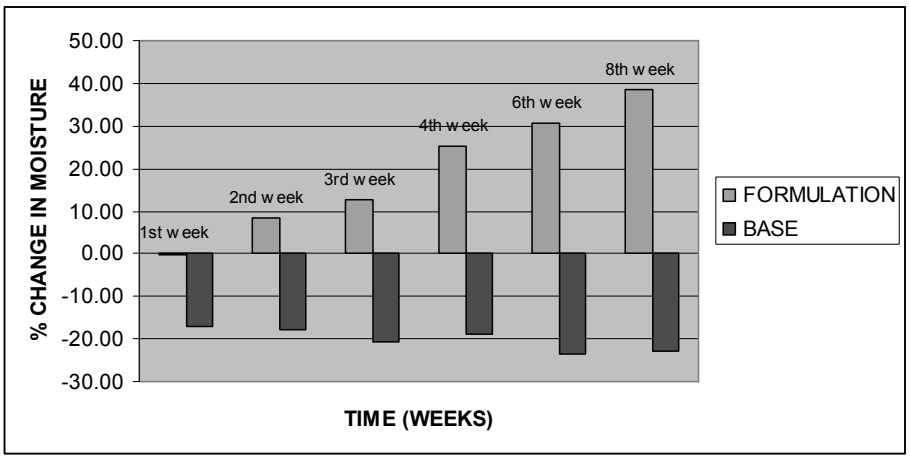

Fig.2. *Percentage of Change in Skin hydration after Application of Base and Formulation*

With the help of ANOVA two way analyses both base and the formulation shown significant effects for skin moisture contents. With paired sample t-test significant differences were observed in skin moisture contents throughout study period except 1st and 2nd weeks. The significant results found with formulation are due to lipids present in green tea i.e. Linoleic acid and α-linolenic acids [14]. Linoleic acid and α-linolenic acids are essential fatty acids which improve skin physiology via their effects on barrier repair by improving the barrier permeability [15]

4.3.6. Trans Epidermal Water Loss (TEWL)

TEWL is *in-vivo* measurement method for testing stratum corneum barrier function of the human skin [17]. The stratum corneum layer of the epidermis is outermost layer of the skin which acts as main barrier. It maintains selective permeability of the substance in and out of the skin [18]. More over glycolipids in the epidermis prevent water loss from the body [19]. On the other hand dermis contains water, ground substance and elastic fibers [16]. Reason behind every morphological change like wrinkles is directly related to loss of collagen which has strong relation with transepidermal water loss. More the epidermal water loss less water is retained by the collagen and results in collagen degeneration. A true action is needed to reverse these changes [20].

There was decrease in TEWL values both by the Base formulation and the Active formulation but more elegant results were observed with the formulation as shown in Fig. 3. With the help of ANOVA it was obvious that the Active formulation revealed significant results while the Base formulation with insignificant results when compared with respect to time. With paired sample test significance in results was observed with the Active formulation except for 1st, 2nd and 4th weeks.

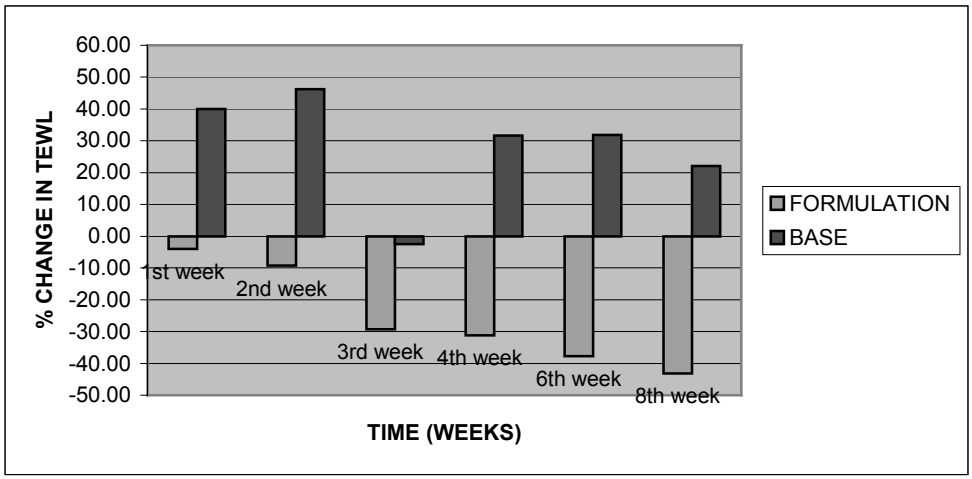

Fig. 3. *Percentage of Change in Values of Trans Epidermal Water Loss (TEWL) after Application of Base and Formulation*

4.3.7 Panel Test

Sensory evaluations of both base and formulation by the volunteers in the form of a questionnaire have been presented in Fig. 4.

Two copies of a questionnaire containing seven questions were prepared and these forms were given to volunteers for sensory evaluation of the two creams i.e. base and formulation. Average points were calculated from the points assigned by each volunteer for each question for both of the creams (base and formulation) and the results were compiled. It was found from average score assigned by the volunteers that the formulation was more aesthetic in all aspects of sensory evaluation by the volunteers.

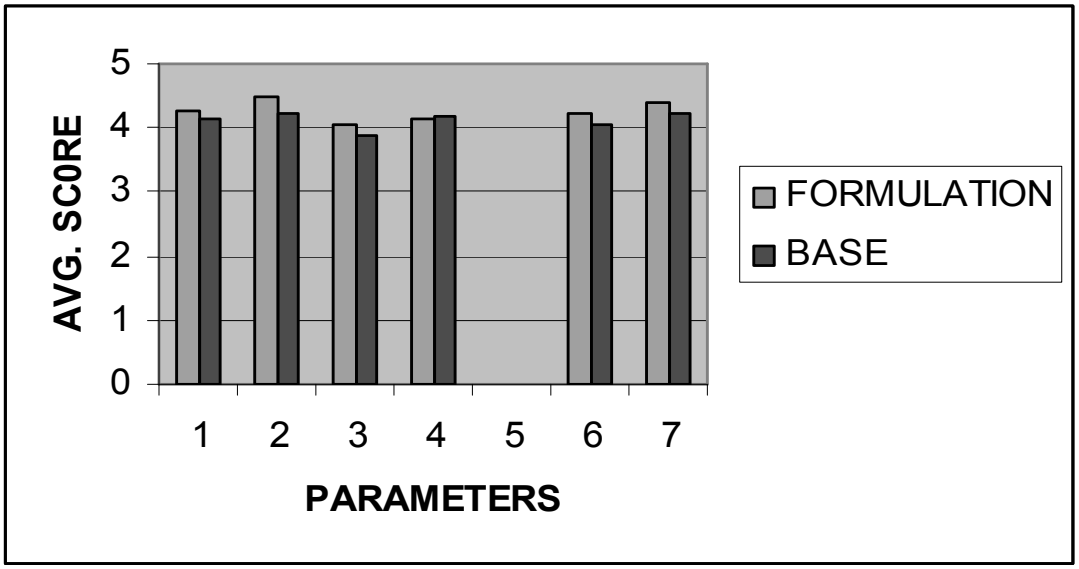

Fig. 4. *Average Values for Panel Test*

Here 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

5. CONCLUSION

It was concluded from the present study that a stable aesthetic formulation of *Camellia sinensis* extract with ideal skin pH range, can be formulated using paraffin oil and ABIL®EM90 as an emulsifier. Both the Active formulation and the Base formulation increased skin hydration level to a significant level but rhythmic results were observed with the formulation. Transepidermal water loss (TEWL) was reduced to a significant level ($p \leq 0.05$) with the Active formulation

over the defined study period 60 days. Hence barrier function of the skin was improved with the Active formulation so discussed.

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Conflict-of-interest policy

Authors don't have any commercial affiliations, or potential conflicts of interest associated with this work submitted for publication.

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