

In Vivo Evaluation of Stable Cream Containing Flavonoids on Hydration and TEWL of Human Skin

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Abstract—Antioxidants contribute to endogenous photo-protection and are important for the maintenance of skin health. The study was carried out to compare the skin hydration and trans-epidermal water loss (TEWL) effects of a stable cosmetic preparation containing flavonoids, following two applications a day over a period of tenth week. The skin trans-epidermal water loss and skin hydration effect was measured at the beginning and up to the end of study period of ten weeks. Any effect produced was measured by Corneometer and TEWA meter (Non-invasive probe).

Two formulations were developed for this study design. Formulation one the control formulation in which no apple juice extract (Flavonoids) was incorporated while second one was the active formulation in which the apple juice extract (3%) containing flavonoids was incorporated into water in oil emulsion using Abil EM 90 as an emulsifier. Stable formulations (control and Active) were applied on human cheeks (n = 12) for a study period of 10 weeks. Result of each volunteer of skin hydration and TEWL was measured by corneometer and TEWA meter. By using ANOVA and Paired sample t test as a statistical evaluation, result of both base and formulation were compared. Statistical significant results ($p \leq 0.05$) were observed regarding skin hydration and TEWL when two creams, control and Formulation were compared. It showed that desired formulation (Active) may have interesting application as an active moisturizing cream on healthy skin.

Keywords—Apple juice extract, TEWL, Corneometer, flavonoids.

I. INTRODUCTION

SKIN is the largest organ of the body, serving as a protective shield against light, injury, heat and infection.

Skin is involved in the regulation of body temperature, water and lipid stores. Structure, texture, thickness, density, hydration, color and shielding properties of skin is changed

with age and vary depending on endogenous and exogenous factors. The nutritional effect of organism effects skin conditions [1].

Flavonoids, a subclass of polyphenols, are secondary plant metabolites found in many consumed fruits and vegetables and, as such, are abundant in a plant rich human diet [2]. Many flavonoids are efficient antioxidant in vitro and the in-vitro antioxidant capacity of a number of fruits and vegetables can be attributed, in part, to flavonoids constituents [3]-[4]. In addition to their putative function as antioxidant, flavonoids can modulate enzyme activity, influence anti-inflammatory pathways and effect cell division [5]. A part from fruits and vegetables, important sources of flavonoids in human nutrition are cocoa, tea and apple [6]-[7].

Moisture retention capability is the basic function of skin-care cosmetics and skin health products. The moisture retention tissue of skin is damaged with age and exposure to external environments. When the water in the cuticle is reduced to $\leq 10\%$, the skin will be dry, non elastic, wrinkled which will quicken skin aging. Thus, water is important for skin and moisture retention is important part of delaying skin aging. Moreover, many natural plant extracts not only possess good moisture retention capability but also have a definite nutritional effect on human skin. Thus natural active plant extracts have been replacing chemosynthetic moisturizers in accordance to the customers demand for naturalness. Among the numerous external factors promoting skin aging, photo-aging produced by ultraviolet (UV) light has been proved to be main factor. Skin exposed to sunlight appears to be 30 year older than exposed skin. Thus sun protection is important method to delay skin aging. UV light is artificially divided into three ranges: (1) UV-A is radiation in 320-400 nm range; (2) UV-B is radiation in 290-320 nm range; (3) UV-C is radiation in 100-290 nm range. Generally UV-B has been blamed for sun burn and some studies indicate that UV-A may cause skin damage. These findings indicate that the development of sunscreens which block UV-A and UV-B rays has great market potential. Therefore in the present study, a stable dermatological formulation containing flavonoids were formulated and applied on human cheeks, compared with control (Without flavonoids) and investigated the skin hydration and Total Epidermal Water Loss (TEWL) effects produced by formulation.

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II. EXPERIMENTAL

A. Materials

Abil® EM 90 was purchased from Franken Chemicals (Germany), Double distilled water was prepared by using distillation plant (IM 100-0.43, IRMECO-GMBH, Germany). Digital pH-meter (WTW, Germany), digital conductivity-meter (WTW, Germany), stability chambers (Sanyo, Japan), water bath (HH-S 21-4, China), electrical balance (Precisa, Switzerland), digital humidity-meter (TES Electronic Corp., UK), centrifuge (Hettich, Germany), mechanical mixer (IKA, Germany), refrigerator (Dalwance, Pakistan), concentrator, Corneometer MPA5 & TEWA meter MPA5 (Courgae + kazaka, Germany), microscopic software (MiniSee, Japan) and SPSS 12.0 were used during the study. Apple was purchased locally.

III. METHODS

A. Extraction Preparation

The basic principle for apple juice production was adopted described by V.L.Bump [8]. Flavonoids from the samples were isolated by methanol formic acid-ddH₂O (MFW; 70:2:28, v/v) solvent [9].

B. Determination of Antioxidant activity

Reduction of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical radicals was used to investigate the radical scavenging potential of the test extracts [10].

C. Formulation Development

For apple juice extract, the oily phase consists of paraffin oil (16%), Bees wax (3%) and emulsifying agent ABIL-EM 90 (2%) was heated up to 75°C±1°C. At the same time, aqueous phase consisting of distilled water was heated to the same temperature. After heating, aqueous phase was added to the oily phase drop by drop. Stirring was continued at 2000 rpm by the mechanical mixer for 15 minutes until complete aqueous phase was added. 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 reduced to 500 rpm for further 5 minutes for complete homogenization; until the emulsion cooled to room temperature [10].

D. Study Design

A total of 12 male healthy volunteers with age ranges 18-25 years were included in this study. Exclusion criteria included smoking, sun bathing, and intake of medication that may influence the outcomes of the study. Participants were advised not to change their habits. No supplements (vitamins, polyphenols) were allowed during study. Single blinded study was designed for the comparisons of two creams i.e. the active formulation containing apple juice extracts and control. Two formulations were named A (Active formulation) and B (Base formulation) and given to the volunteers with instructions of application. All the skin tests were performed at 21±01°C and

40±2% relative humidity conditions and all the measurements were taken by a single expert [10].

E. Application of cream

The experiments were carried out on the cheeks of volunteers. On the first day; patch test (Burchard test) was performed on the forearms of each volunteer to determine any possible reactions to the emulsions. Each volunteer was provided with two creams. One cream was Base and the other 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 10 weeks. Every volunteer was instructed to come for measurement on 1, 2, 3, 4, 6, 8 and 10th week.

F. Ethical standards

This study was approved by the Board of advance study and Research (BASR), The Islamia University of Bahawalpur and Institutional ethical committee. The Reference No. is Ref No: 8660/Acad.

G. Skin hydration and Trans-epidermal water loss

Skin hydration (arbitrary units) was determined by corneometer (Corneometer MPA 580, Courage and Khazaka Electronics Germany) and TEWL (g.h.m²) was measured by using a TEWA-Meter MPA 580 (Courage and Khazaka Electronics Germany).

IV. RESULTS AND DISCUSSION

A. Evaluation of cream

Stability of creams (Base and formulation) was evaluated on different storage conditions i.e. 8°C, 25°C, 40°C and 40°C + 75% RH for 28 days. Centrifugation and accelerated temperature conditions are very important parameters for stability of creams [11]. No phase separation was observed during the stability study of creams. As elevated temperature cause change in viscosity, partition and solubility of molecules between two phase. But it has found that lipophilic surfactant is more stable at elevated temperature [12]. No liquefaction is observed throughout the study period of 28 days.

V. IN-VIVO CHARACTERIZATION OF CREAMS FOR SKIN HYDRATION AND TEWL

A. Burchard Tests (Patch Tests)

On the first day of skin testing, patch tests were performed on the 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 was 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. After 48 hours, scores were recorded for the presence of erythema (skin redness) using a scale with 4 points from 0 to 3. Where 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 Table 1 [13].

TABLE I
 SCORE GIVEN BY VOLUNTEERS TO BASE AND FORMULATION ON THE BASIS OF
 ITCHING/IRRITATION*

SCORE		0	1	2	3
No of volunteer	Base	7	4	1	0
	Formulation	8	3	1	0

B. Skin Moisture

In this study controlled formulation improved the moisture content of the skin to some extent but formulation rhythmic increase in water contents of the skin and the results of the average percentage occurred in the skin moisture content are shown in fig.1.

With the help of ANOVA two way analyses both control and formulation shown significant effect for skin moisture content. With the paired sample t test, significant difference was observed in skin moisture content throughout the study period.

Vitamin C is the most important vitamin in apple with a mean concentration of 120mg/kg. The observed variation in compound concentration can be large, e.g. for vitamin C values between 30 to 250mg/kg are reported [14]. Vitamin C has the advantage of stimulating derm fibroblasts for the synthesis of collagen. As the collagen level is increased, the hydration level also improved [15].

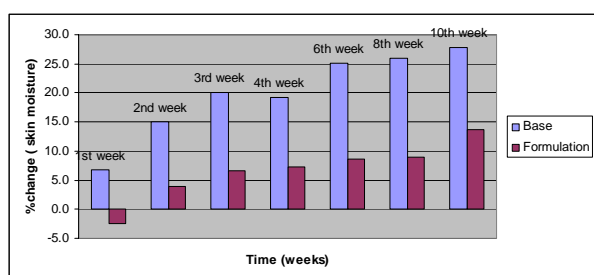


Fig. 1 Percentage of change of skin hydration after application of base and formulation

C. Trans-epidermal water loss (TEWL)

In this study, it was found that there were variations in TEWL values after the application of Base. On the 1st and 2nd week it was decreased while on the, 3rd, 4th, 6th and 8th week it was increased. In case of formulation there was gradual decrease in TEWL throughout the study period.

With the help of ANOVA test, it was found that change in TEWL produced by formulation were significant ($p \leq 0.05$) with respect to time, whereas applying ANOVA test to the base it was found that changes in TEWL values produced were insignificant ($p \geq 0.05$) with respect to time.

With the help of paired sample t-test it was found that there was insignificant ($p \geq 0.05$) variation in TEWL with respect to Base and Formulation for the 1st two weeks while from 4th week to the 10th week of study significant ($p \leq 0.05$) differences were observed between the TEWL values of Base and the Formulation. The underlying mechanism is not known; however it is proved scientifically that flavanols mediated increase in cutaneous blood flow likely contributes to an improvement in skin appearance.

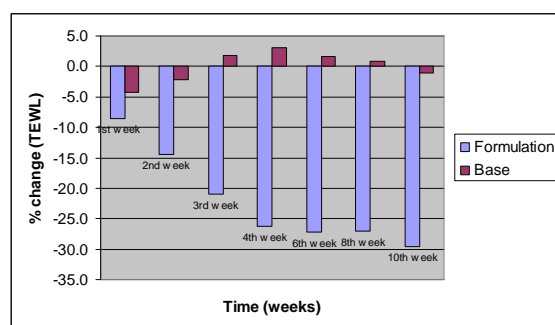


Fig. 2 percentage of change in values of Trans Epidermal Water Loss (TEWL) after application of control and formulation

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