Asian Journal of Chemistry; Vol. 23, No. 2 (2011), 903-906

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in

Effects of Antioxidants and Flavonoids of Sea Buckthorn on Skin Whitening and Skin Erythema

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(*Received*: 3 May 2010; *Accepted*: 15 October 2010) AJC-9192

The aim of this study is to determine the effects of newly formulated W/O emulsion (cream) of Sea Buckthorn *versus* its vehicle (base) as control on skin melanin and skin erythema. Sea Buckthorn extract was entrapped in w/o emulsion. The newly formulated base and formulation (containing 1 % concentrated extarct of Sea Buckthorn) were applied to the cheeks of 21 healthy human volunteers for 8 weeks. The base showed insignificant ($p > 0.05$) effects while the formulation showed statistically significant ($p \leq 0.05$) decrease in skin melanin content. Skin erythema was significantly ($p \leq 0.05$) reduced by the formulation.

Key Words: Melanin, Sea Buckthorn, Flavonoids.

INTRODUCTION

Melanin, a biological pigment found in skin is produced by melanocytes in the stratum basal of epidermis. Melanin is synthesized by the conversion of amino acid tyrosine to dopaquinone catalyzed by enzyme tyrosinase. Overproduction and accumulation of melanin causes skin problems such as freckles, melasma and age spots. It has been reported that many cosmetic agents inhibit tyrosinase activity or block melanogenic pathways leading to skin whitening[1]. Conventional whitening agents, such as hydroquinone and kojic acid are effective depig-menting agents. However they have some safety concerns with long-term exposure. Natural extracts provide an idea to develop new products for hyperpigmentation[2]. It has been studied that the natural compounds such as flavonoids inhibit melanogenesis without melanocytotoxicity. Flavonoids such as quercetin, kaempferol and myricetin are chemically polyphenolic antioxidants naturally present in vegetables, fruits and beverages. The common flavonol, quercetin (Fig. 1), was previously isolated as the major tyrosinase inhibitor by chelating its copper[3,4].

Sea Buckthorn (*Hippophae rhamnoides* L) is a deciduous, dioecious plant with numerous greenish-yellow flowers and bright orang, globular, ellipsoid fruit belongs to family Elaeagnaceae[5]. Flavonoids are present in all parts of Sea Buckthorn. The main flavonoids in seabuckthorn are isorhamnetin, quercitin, myricetin and kaempferol[6]. Sea Buckthorn has been used for the treatment of radiation damage, inflammation and burns in Chinese folk medicines as well as in the treatment of skin disorders such as eczema, psoriasis, lupus erythematosus and dermatosus[5]. Generally the medical application and development of Sea Buckthorn flavonoids compound is one of the necessary and important steps for the broad use of Sea Buckthorn[6]. This study depicts Sea Buckthorn actives as topical treatments for hyperpigmentation disorders and skin erythema.

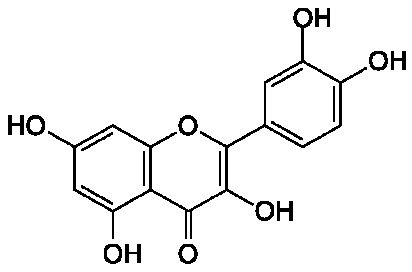

Fig. 1. Chemical structure of quercetin

EXPERIMENTAL

For the formulation of emulsions/creams (applied in the study) Sea Buckthorn berries were purchased from Pak Sea Buckthorn International Skardu, Pakistan. ABIL-EM90 (Emulsifying agent) was purchased from Franken Chemical (Germany) and Methanol, *n*-hexane and paraffin oil were purchased from Merk KGaA Darmstadt (Germany). Ethanol was taken from BDH England.

Mexa meter MPA5 (Courage + Khazaka, Germany), HPLC 20A (Shimadzo Japan) and SPSS 12.

Determination of flavonoids: A very simple, rapid and efficient method of Zu *et al.*[7] with slight modification was applied for the determination of flavonoids (quercetin, kaempferol and isorhamnetin) in Sea Buckthorn berries. The sample was ultrasonified at high frequency. 70 % ethanol was used as extraction solvent at a temperature of 30 ºC for 1 h. Water -methanol-acetonitrile with 1 % acetic acid was used as mobile phase. Diode array detection was carried out at 368 nm wavelength for the detection of flavonoids. Flavonoids were determined with successful recoveries of 92, 81 and 85 % of quercetin, kaempferol and isorhamnetin, respectively.

Antioxidant activity of Sea Buckthorn: The free radical scavenging activity of Sea Buckthorn was determined in accordance to Blois method[8] using DPPH (1,1-diphenil-2-picrylhydrazyl) which is a stable free radical. Equal volumes of diluted extract were mixed with an equal volume of DPPH 6×10^{-3} M in absolute ethanol and the obtained mixtures were kept at room temperature for 20 min. Then, the absorption of the mixtures at 517 nm was taken, in comparison with the control solution (maximum absorption). Vitamin C was used as standard. The activity of free radicals was calculated in percentage inhibition according to the following relation:

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

The free radical scavenging activity of Sea Buckthorn was 80 % in comparison to the standard. It may due to the presence of natural antioxidants in Sea Buckthorn such as vitamin C, flavonoids, carotenes and tocopherols.

Emulsions (creams): In this study the products studied were newly formulated W/O emulsions (base and formulation) which were found stable after evaluating for pH, electrical conductivity, centrifugation, phase separation, temperature stability tests at 8 ± 0.1 ºC (in refrigerator), 25 ± 0.1, 40 ± 0.1 and 40 ± 0.1 ºC with 75 % RH (in incubator) and physical characteristics *i.e.*, colour, creaming and liquefaction[5].

Study design for product evaluation on skin: One-sided blind study was designed with placebo control in the month of August to September. Twenty one male healthy human volunteers who signed the informed consent, with age range of 20-35 years were selected. All the skin tests were performed at 21 ± 01 ºC and 40 ± 2 % relative humidity conditions and all the measurements were taken by a single expert.

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 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 measurements.

Ethical standards: This study was approved by the board of advance study and research (BASR), The Islamia University of Bahawalpur and Institutional ethical committee. The Reference No. is 1663.

Burchard tests (Patch tests): On the first day of skin testing, patch tests were performed on the forearms of each volunteer. A 5 cm × 4 cm 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 5 cm × 4 cm marked regions separately on each forearm. The regions were covered with the surgical dressing after application. The patches were removed after 48 h and the forearms were washed with physiological saline[9]. After 48 h, scores were recorded for the presence of erythema (skin redness) using a scale with 4 points from 0-3. 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.

TABLE-1
SCORE GIVEN BY VOLUNTEER TO BASE AND FORMULATION ON THE BASIS OF ITCHING/IRRITATION*

Score		0	1	2	3
No. of volunteer	Base	16	3	2	0
	Formulation	14	4	3	0

*No severe erythema occurred in any of volunteer, mild erythema occurred in 2 and 3 volunteers, moderate erythema occurred in 3 and 4 volunteers, whereas no erythema occurred in 16 and 14 volunteers for both base and formulation, respectively.

Panel test: Every individual was provided with a performa prepared previously to test the sensory values of creams. This performa 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 performa was asked to be completed independently by each individual at the end of study period. From the average reply of volunteers it was concluded that base and formulation were felt well on the skin, produced pleasant feeling on application to skin and no irritation on the skin in both cases *i.e.*, base and formulation, as these were assigned 0.00 point for irritation by all the volunteers. Shine on skin was more for formulation. This was expected since the formulation contained essential fatty acids. Similarly, the formulation led to more softness of the skin than base.

It was found from paired sample t-test that there was an insignificant difference between the average points of sensitivity for base and formulation. It was concluded that there was no immense variation between base and formulation regarding the sensory evaluation. Both of the creams have similar performance from the sensory point of view.

Mathematical analysis: The percentage changes for the individual values of skin melanin and skin erythema, taken every week, of volunteers were calculated by the following formula;

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

where; A = individual value of any parameter of 1st, 2nd, 3rd, 4th, 6th or 8th week. B = zero hour value of that parameter.

Statistical analysis: The measured values obtained for skin melanin and skin erythema were analyzed using SPSS 12.0 on the PC (paired samples t-test for variation between the two preparations; two-way ANOVA for variation between different time intervals while using a 5 % level of significance for both skin parameters.

RESULTS AND DISCUSSION

Skin melanin content: The per cent change occurred in the skin melanin content before and after applications of base and formulation have been presented in Table-2.

Skin erythema: The per cent changes occurred in the values of skin erythema before and after applications of base and formulation have been given in Table-3.

Skin melanin content: In this study, the effect of base and the formulation on skin melanin was examined. The change in skin melanin was measured for 8 weeks at different time intervals in each individual. After application of base it was found that there was variation in the melanin contents of the skin throughout the study period. At 1st, 2nd and 3rd week there was decline in skin melanin contents while during 4th, 6th and 8th week there was increase in skin melanin. In case of formulation there was continuous decline in the melanin contents throughout the study period (Table-2).

With the help of ANOVA test it was found that the base produced insignificant ($p > 0.05$) effects with respect to time.

When ANOVA test was applied for formulation it was concluded that formulation produced significant ($p \leq 0.05$) effects with respect to time.

With the help of paired sample t-test, significant differences were observed between the melanin effects of base and the formulation from the 2nd week till the end of study period.

It has been established by a number of studies that flavonoids especially quercetin is a potent tyrosinase inhibitor so the formulation reduced the skin melanin content[10]. Flavonoids are present in all parts of Sea Buckthorn where fresh fruits contain 854 mg/100 g while dried leaves contain 3888 mg/100 g of flavonoids. The main flavonoids in Sea Buckthorn are isorhamnetein, quercetin, myricetin and kaempferol[6].

Skin erythema: In this study skin redness (erythema) was constantly monitored throughout the study period of 8 weeks for base and formulation. It was found that there was an irregular slight increase in the erythema contents after the application of base throughout the study period, whereas after the application of formulation erythema contents were decreased throughout study period (Table-3).

With the help of ANOVA test it was found that the base produced insignificant ($p > 0.05$) effects on skin erythema with respect to time where as in case of formulation, it was found that formulation produced significant ($p \leq 0.05$) effects on skin erythema with respect to time.

With the help of paired sample t-test, significant differences were observed between the erythema effects of base and the formulation from the 3rd week of study period.

Damage of DNA, cellular lipids and proteins by free radicals are considered to be the cause of erythema. Carotene especially β carotene and tocopherols are effective free radicals scavengers[11]. Seeds and berries of Sea Buckthorn have sufficient amount of tocopherols (vitamin E). The concentration of tocopherols and tocotrienols ranges from 100-300 mg/1000 g in seeds and 110-150 mg/1000 g of berries. Yellow-orange colour of the berries is due the presence of carotinoids. Carotinoids in seeds are present in a concentration of 1/20-1/5 to that of berries[12].

Conclusion

From the present findings it is concluded that a stable topical cream (W/O emulsion) containing Sea Buckthorn fruit extract can produce a pronounced decrease in melanin content of the skin showing that the formulation has skin whitening effects. The formulation was observed to decrease skin erythema significantly which shows that the formulation has antipsoriasis affects. A targeted study needs to be conducted in future in patients with freckles/melasma and psoriasis so that actual potential of this very plant against these disorders can be explored.

ACKNOWLEDGEMENTS

The authors thank to Higher Education Commission of Pakistan for financial support, The Department of Pharmacy, The Islamia University of Bahawalpur for moral support and Hafiz Nasrullah (Abbott Laboratories Pakistan) for determination of flavonoids contents.

TABLE-2
PERCENTAGE OF CHANGE IN SKIN MELANIN CONTENT AFTER APPLICATION OF BASE AND FORMULATION

	Values of skin melanin content (mean ± SEM)					
Time (week)	1st	2nd	3rd	4th	6th	8th
Base	-0.40 ± 0.99	-0.49 ± 1.13	-0.20 ± 0.94	2.60 ± 0.74	2.67 ± 0.90	1.93 ± 1.76
Formulation	-4.17 ± 0.91	-6.81 ± 1.39	-12.28 ± 1.95	-17.81 ± 2.73	-22.25 ± 2.60	-28.04 ± 3.21

TABLE-3
*PERCENTAGE OF CHANGE IN VALUES OF SKIN ERYTHEMA AFTER APPLICATION OF BASE AND FORMULATION

	Values of skin erythema (mean ± SEM)					
Time (week)	1st	2nd	3rd	4th	6th	8th
Base	8.99 ± 6.25	7.30 ± 6.96	7.01 ± 7.47	7.96 ± 7.26	10.23 ± 9.20	4.20 ± 5.92
Formulation	-2.72 ± 1.56	-4.98 ± 1.12	-6.64 ± 1.29	-9.90 ± 1.26	-13.24 ± 1.51	-15.47 ± 2.30

*Per cent change values are the average of 10 volunteers calculated by using the formula, $\text{Change}\ (\%) = \left[\frac{(A - B)}{B}\right] \times 100$. Here; A = individual value of any parameter of 1st, 2nd, 3rd, 4th, 6th or 8th week. B = zero hour value of that parameter.

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