



## ABATEMENT OF DETRIMENTAL EFFECTS OF PHOTOAGING BY *PRUNUS AMYGDALUS* SKIN EXTRACT

MONIKA KATYAL SACHDEVA<sup>1</sup>, TARUNA KATYAL<sup>2</sup>

College Of Pharmacy, Qassim University, KSA, I.S.F. College Of Pharmacy, Moga, Punjab, India

Received: 29 July 2010, Revised and Accepted: 02 Sep 2010

### ABSTRACT

*Prunus amygdalus* is used extensively in cosmetic formulation owing to its copious properties. In this investigation herbal cosmetic formulation comprising of *Prunus amygdalus* skin extract was prepared and evaluated for the protection of skin from solar ultraviolet induced photoaging. To investigate the antiaging activity of prepared formulation the UVB- induced oxidative stress was generated in mice and protective effects were observed after topical treatment of prepared formulation ( 0.2 mg cm<sup>2</sup> day<sup>-1</sup> ), 2 hrs prior to UV exposures. Biochemical investigations (lipid peroxidation and glutathione) were carried out to evaluate the photoprotective effect of prepared formulation. The change in level of moisture content in formulation treated mice skin group as compared to UV irradiated control mice group was also estimated. The prepared formulation treated mice skin groups showed stronger antioxidant activity by significantly decreased and increased MDA and GSH level respectively as compared to irradiated control group. Remarkable difference could be seen in moisture content in formulation treated mice skin as compared to irradiated control and thus it's confirmed from moisture content and biochemical investigations that topical application of *Prunus amygdalus* skin extract containing formulation possess antiwrinkle properties.

**Keywords:** *Prunus amygdalus* skin, Effect of photoaging.

### INTRODUCTION

There are a numbers of factors responsible for cutaneous aging. Apart from genetically programmed cell aging, different external aggressor such as stress, pollution and the most common solar radiations can accelerate this phenomenon.<sup>1</sup> The aging induced by UV radiations is known as photoaging which is characterized by wrinkles, laxity, uneven pigmentation, leathery appearance.<sup>2</sup> This worsen photoaged skin is the result of excessive production of reactive oxygen species( ROS) which is consider to play important role in cutaneous pathology.<sup>3</sup>

In order to combat various deleterious effects produced by ROS generated from ultraviolet radiation, human body has built-in antioxidant mechanism to suppress uncontrolled free radicals but unfortunately these defense mechanisms may at times fail due to overwhelming production of toxic radicals, the depletion of compounds used in the skin's defence system, or a combination of both.<sup>4</sup> So, to overcome this problem the development of a novel agent that can minimize the effects of ROS and delay cutaneous aging has been the quest of the cosmeceutical industries.<sup>5</sup>

*Prunus amygdalus*, commercially known as almonds is a natural product whose seeds are rich in polyphenolic compounds especially flavanoids and phenolic acids.<sup>6,7</sup> In the past decade, the antioxidant activity of various plant phenolic, flavanoids, carotenoids, catechins, polyphenolic compounds and plant extracts have demonstrated the ability to suppress UV -induced adverse effects.<sup>8,9,10</sup> Hence, this study was undertaken to evaluate antioxidant and antiwrinkle activities of the skin extract of *Prunus amygdalus* in experimentally -induced photoaging.

### MATERIALS AND METHODS

#### Collection and identification of plant material

Seeds of *Prunus amygdalus* were collected from the local market in the month of October-November. The plant material was authenticated on the basis of taxonomic characters and by direct comparison with the herbarium specimens available at the Museum-cum herbarium of Department of Botany, Dr. H.S. Gour University, Sagar, M.P, India.

#### Preparation of plant extract

Seeds of *Prunus amygdalus* were soaked in hot water for 4-5 hrs. The seed coat were removed from white embryo. Removed skin of *Prunus amygdalus* was shade dried, coarsely powdered using a grinder and stored in an air tight, light -resistant container for further use.

#### Extraction

The skin powder was defatted with hexane using a soxhlet apparatus. The defatted marc was further soxhlet- extracted with methanol and the extract obtained was concentrated using a rotary flash evaporator. The extract yield was 14.12% w/w on dry weight basis, and it was stored in a vacuum desiccators for pharmacological evaluation.

#### Preparation of herbal formulation

The extract (0.5g) was mixed with 9.5 g of simple ointment in a porcelain tile and transferred to a tightly closed amber colored container. For topical administration, 5% w/w ointment was prepared in simple ointment base I.P.

#### Selection of animals

Female Lacca mice weighing 15-25 g were used. The animals were housed in plastic bottom cages where they were allowed free access to standard animal feed and water.

The hairs on demarcated area of approximately 4 cm<sup>2</sup> on the dorsal surface of mice were removed using a rose anne french hair removing cream. The mice were observed for 48 hrs and those showing any abnormal hair growth or any reaction to the cream were excluded. A hair removing cream was preferred over a shaving blade to minimize free radical production due to trauma from the blade.

#### UV Light Exposure Conditions and Development of Photo Ageing

Mice were divided into groups of 24 animals each. The first group of 24 animals, neither received UV exposure nor any treatment, was the un-irradiated control. This set of animals were kept to account for any oxidative stress contributed by the depilatory (hair removing cream) which was applied every third day on all animals of all groups. The second group of 24 animals receiving only UV exposure of 5 min. twice a day was treated as irradiated control. The further groups each consisting of 24 mice received both UV radiations and prepared formulation treatment. The treatment was given 2 hrs. Prior to UV exposure.

#### Treatment Protocol

All Animals were kept inside the solar simulator (designed in the laboratory and fitted with UV lamp) at a distance of 40 cm from the UV light source (UV Bulb: Ultra vitalux 300 W waton (R), Germany. The bulb gave the full spectrum of UV radiation i.e. 260-400 nm, simulating the full solar spectrum.) The UV exposure was

controlled by the time of exposure. Exposures were given daily twice for 5 min. For the mice receiving topical formulation treatment, the dorsal skin was treated with prepared formulation ( 0.2 mg cm<sup>2</sup> day<sup>-1</sup> ), 2h prior to each UV radiation exposure.. Animals were treated for 3, 7, 11 and 15 days following which the skin was excised and used for biochemical estimation and moisture content.

### Biochemical investigation

#### Lipid peroxidation assay<sup>11</sup>

The levels of lipid peroxidase were estimated in un-irradiated control group, irradiated control group and groups received both UV radiations and prepared formulation treatment. 1 g sample of treated skin was taken. To this 9 ml of 1.15% KCl was added and homogenized while keeping temperature of the tissue below 5°C to prevent free radical generation due to heat production. Homogenization was continued until a viscous; turbid mixture with no solid particles was formed.

To a sample (0.2 mL) of 10% (w/v) tissue homogenate, 0.2 mL of 8.1% sodium lauryl sulphate and 1.5 mL of 20% acetic acid solution (pH adjusted to 3.5 with sodium hydroxide) were added. Then 1.5 mL of 0.8% aqueous solution of thiobarbituric acid (TBA) was added. The mixture was made up to 40 mL with distilled water and then heated on a water bath at 95°C for 60 minutes. After cooling with tap water 1.0 mL of distilled water and 5.0 mL mixture of n-butanol and pyridine (15:1) were added to the above mixture which was shaken vigorously. After centrifugation at 4000 rpm for 10 minutes, absorbance of the organic layer was recorded at 532 nm. The levels of lipid peroxidase were expressed as nanomoles (nmole) MDA (malondialdehyde) per gm of tissue.

#### Estimation of glutathione (GSH)<sup>12</sup>

Glutathione reduces H<sub>2</sub>O<sub>2</sub> directly to water or react directly with the free radicals such as O<sub>2</sub><sup>-</sup>, OH<sup>-</sup>, O<sup>-1</sup> by a radical transfer process which yield thiol radicals. This thiol radical or the sulfhydryl group present in glutathione forms a colored complex with DTNB which is measured spectrophotometrically at 412 nm.

The promptly excised skin was kept in chilled 0.9% NaCl. After washing with saline, 500 mg of skin tissue was homogenized with 5ml of 10% TCA using Teflon glass tissue homogenizer (Remi India) and centrifuged at 3000 rpm (4°C) for 10 min. The supernatant diluted 10 fold in phosphate buffer and kept on ice was used for study of glutathione.

The reaction mixture contained 2ml. Phosphate buffer (5.225 gm in 100 ml D.W.; PH adjusted to 8.4), 100 ml of sample and 500 ml of 0.002% DTNB (2mg DTNB in 1% sodium citrate). To this reaction mixture 400 ml of distilled water was added and absorbance measured at 412. Change in absorbance was measured and the level of GSH was expressed as mg/gm of tissue.

#### Moisture content<sup>13</sup>

##### Procedure

For measuring the moisture content, the fresh skin samples were weighed accurately. The samples were kept for drying until a constant weight was observed which was found to be 24h on an average. Moisture content was calculated by the following formula:

$$\text{Moisture content (\%)} = \frac{\text{Wt. of skin before drying} - \text{wt. of skin after drying}}{\text{Wt. of skin before drying}} \times 100$$

### Statistical analysis

The results were expressed as mean  $\pm$  SEM of 6 animals in each group. The data were statistically evaluated by one - way Anova , followed by Dunnet's test for comparison of test group with control. Values of p $\leq$ 0.05 were considered as statistically significant.

### RESULTS AND DISCUSSION

In the present biochemical investigation the elevated level of end product lipid peroxidation in mice skin exposed to UV radiations were observed. The increase in MDA level in skin tissue suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanism to prevent formation of excessive free radicals. Pretreatment with prepared formulation (0.2 mg cm<sup>2</sup> day<sup>-1</sup> ) significantly reversed these changes by decreasing MDA level from 764 $\pm$ 6.89 to 216 $\pm$ 15.56 nanomoles MDA/gm of tissue in 15 days of treatment period. Prepared formulation was also found to better than marketed formulation in decreasing MDA level as compared to irradiated control but the effect could be seen better than marketed formulation only after 7 days of treatment period. (Table 1)

The level of glutathione was significantly low in UV induced oxidative stress mice when compared to un- irradiated control. The mice ground pretreated with prepared formulation showed remarkable increase in GSH level. The elevation in GSH from 71 $\pm$ 5.18 to 176 $\pm$ 19.0  $\mu$ g GSH /gm of tissue could be observed in formulation treated mice skin after 15 days of treatment period.(Table 2)

The moisture content of the skin is greatly influenced by ground substances and the loss of water may be responsible for wrinkling and laxity of the skin accompanying cutaneous ageing. The moisture holding capacity of ground substances gradually decreases with the advancing age and the effect could be worsen with elevated level of free radicals which resulting in skin premature ageing.

Present study also showed the same effect by decreasing Weight of irradiated mice skin (in mg) from 623 $\pm$ 14.66 to 496 $\pm$ 18.2. The prepared formulation could retain moisture as the weight retained from 496 $\pm$ 18.2 to 601 $\pm$ 15.50(in mg) in formulation treated mice skin group in 15 days of treatment period.( Table 3)

The data clearly indicated that the formulation comprising of *Prunus amygdalus* extract capable of scavenging the free radicals by showing the decrease and increases of MDA and GSH level respectively and also enhanced the moisture content in prepared formulation treated mice group as compared to irradiated control mice group. This may be possible because of presence of polyphenolic constituents especially flavonoids in *Prunus amygdalus* extract in prepared formulation. Therefore being rich in phenolic constituents, almond skin can be considered as a value-added by product for elaborating topical antioxidant ingredient. Further bioactivity guided fractionation studies for isolation and identification of active principles responsible for antioxidant and moisture retained properties is underway.

### CONCLUSION

It is concluded from present findings that *Prunus amygdalus* extract containing formulation may also have a role in preventing the adverse effects of photoaging attributable to enzymic and nonenzymic mediated dermal damages and may contribute as a new antiwrinkle cosmetic ingredient for protection from UVB-induced skin damage .

**Table 1: Results of lipid peroxidation assay**

S. No.	Group	Experiment duration 3-15 days			
		Nanomoles MDA/gm of tissue $\pm$ S.D. (n= 6)			
		3 day	7 day	11day	15 day
I	Un- Irradiated Control	138 $\pm$ 4.29	150 $\pm$ 3.26	180 $\pm$ 3.79	205 $\pm$ 5.25
II	Irradiated Control	224 $\pm$ 11.06	389 $\pm$ 17.45	599 $\pm$ 15.33	764 $\pm$ 6.89
III	Prepared formulation	192 $\pm$ 11.61	301 $\pm$ 11.20	228 $\pm$ 16.73	216 $\pm$ 15.56*
IV	Marketed formulation	189 $\pm$ 3.39	289 $\pm$ 21.31	368 $\pm$ 16.95	471 $\pm$ 11.29

\*Values are more significant (p $\leq$ 0.05) as compared to irradiated control.

Table 2: Results of glutathione estimation

S. No.	Group	Experiment duration 3-15 days			
		µg GSH /gm of tissue ± S.D. (n=6)			
		3 day	7 day	11 day	15 day
I	Un-irradiated Control	212±18.24	197±11.17	190±18.17	188±14.90
II	Irradiated Control	159±16.08	125±14.15	99±10.19	71±5.18
III	Prepared formulation	176±11.24	177±11.12	161±17.19	176±19.09*
IV	Marketed formulation	181± 14.02	164±5.62	144±15.50	129±11.31

\*Values are more significant (p<0.05) as compared to irradiated control

Table 3: Results of determination of moisture content

S. No.	Group	Experiment duration 3-15 days			
		Weight of skin in mg ± S.D. (n=6)			
		3 day	7 day	11 day	15 day
I	Un- Irradiated Control	662±19.00	680±9.88	672±10.24	623±14.66
II	Irradiated Control	626±10.01	603±12.91	547±12.71	496±18.2
III	Prepared formulation	632±12.01	631±16.71	611±13.12	601±15.50*
IV	Marketed formulation	639±17.99	646±13.02	578±10.20	587±19.56

\*Values are more significant (p<0.05) as compared to irradiated control.

#### REFERENCES

1. Evridiki TN, Rachel EBW, Christopher EMG. Photoaging: the darker side of the sun. Photochem .Photobiol. Sci. 2006; 5:160-164.
2. Gilchrest BA, Massachusetts MD. Skin aging and photoaging :an overview. J Am Acad Dermatol 1989 ; 21: 610-613.
3. Ansari KN. The free radicals – The hidden culprits-An update. Ind J Med Sci 1997; 51: 319-336. 4. Svobodova A, Psotova J, Walterova D .Natural phenolics in the prevention of UV –induced skin damage. A review. Biomed Papers 2003; 147(2):137-145. 10.
4. Kligman AM . Cosmeceuticals as a third category. Cosmet Toilet. 1998; 113: 33-40.
5. Milbury PE, Chen CY, Dolnikowski GG, Blumberg JB. Determination of flavonoids and phenolics and their distribution in almonds. J agri food chem. 2006;54(14):5027-33.
6. Kim YH, Yang HE ,Park BK, Heo MY, Jo BK, Kim HP .The extract of the flower of *prunus persica* ,a new cosmetic ingredient ,protects against solar ultraviolet –induced skin damage in vivo . J.Cosmet Sci. 2002 ;53(1):27-34.
7. Wijeratne SS,Abou-Zaid MM, Shahidi F. Antioxidant polyphenols in almonds and its coproducts. J. Agric Food Chem 2006 ; 54(2) :312-8.
8. 9.Monagas M,Garrido I, Lebron –Aguilar R ,Bartolome B,Gomez –Cordoves C . Almonds prunus dulcis (mill) skin as a potential source of bioactive polyphenols. J Agric food chem.. 2007;55(21) :498-507.
9. Kim BJ, Kim J.H ,Heo M.V. Biological screening of 100 plant extracts for cosmetic use . Int. J. Cosmetic Sci 1997; 19:299-307.
10. 11. Ohkawa H, Ohishi N ,Yagi k. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979; 95: 351-358.
11. 12. Ellman GL . Tissue sulfhydryl group . Arch Biochem Biophys. 1959; 82: 70-77.
12. Jung JW, Cha SH, Lee SC ,Chun IK , Kim YP Age related changes of ceraten content in the rate skin. J. Dermatol Sci. 1997 ;14 : 12-19.