International Journal of ChemTech Research
ISSN : 0974-4290
Vol.1,No.1,pp 88-95, Jan – March 2009

Formulation and Evaluation of Cream of *Azadirachta indica* leaves extracts on Skin Renewal rate

Kamlesh J. Wadher[1*], C. L. Lakhotia[2] and M. J. Umekar[1]

[1]S. K. B. College of Pharmacy, New Kamptee, Nagpur,M.S.,India.

[2]Department of Pharmaceutical Sciences, RTM Nagpur University, Nagpur, M.S.,India.

* Corres.author : kamleshwadher@gmail.com

Abstract:

Azadirachta indica (Neem) is one of the most popular auspicious and well known tree which is more extensively studied for its pharmaceutical and clinical properties. Three creams containing 0.5 %, 1.0 % and 2.0 % w/w of hydroalcoholic macerate extracts of leaves of neem were prepared. Creams were prepared in model FAPG base. Students t test shows that 1.0 % and 2.0 % hydroalcoholic macerate creams shows increase in skin renewal rate , which is significant at $p<0.005$ level of significance. While 0.5 % hydroalcoholic macerate extracts cream is significant at $p<0.05$ level of significance.

Key words: *Azadirachta indica* (Neem), hydroalcohol macerate extracts, FAPG base, cream.

Introduction

A variety of biological activity of Azadirachta indica (Neem) has been reported in literature. Literature revealed antifertility[1], hypoglycaemic[2], wound healing[3] and analgesic[4]activity of *Azadirachta indica*.

In present investigation FAPG base was employed for the preparation of creams of 0.5 %, 1.0 % and 2.0% w/w hydroalcoholic macerate extracts of leaves of neem and the prepared creams were tested for their anti aging activity.

Experimental

Materials and method:

Dansyl chloride was purchased from sigma chemicals Japan. An occlusive adhesive patch was purchased from Johnson and Johnson, India. All the other chemicals used were of AR grade. Spectrofluorometer Jobin Yvou, Japan was used for analysis**.**Fresh healthy neem leaves were collected from vicinity of Nagpur University campus,

Nagpur; India and allowed to dry in a shade under normal environmental condition for about one week and then crushed it into small pieces by hand.

Cold maceration of dried neem leaves:

About 100 g of coarsely grounded leaves were steeped in hydroalcohol (1:1) for one week at ambient temperature and then filtered. This extraction was repeated with another fresh portion of hydroalcohol (1:1) for about one week. The hydro alcohol macerate extracts were combined and evaporated up to dryness, dark brown coloured product was obtained**.**

Formulation of cream of hydroalcoholic macerate extracts.

FAPG base contains 5 % steric acid, 20 % steryl alcohol and 75 % propylene glycol was used. The steric acid and steryl alcohol were heated up to 52^{O}C. Propylene glycol containing 0.5 g ,1.0 g ,and 2.0 g of dried hydroalcohol macerate extracts were also heated up to 52^{O} C .The two phases were then mixed and triturated thoroughly in a mortar until a thick viscous mass obtained. It was then allowed to cooled and congealed.A series of modified FAPG base were prepared on trial and error basis following the above said procedure. The series of prepared cream are depicted in Table1.

Table 1
Formulation of various creams of FAPG base:

Type	Stearic Acid %	Stearyl Alcohol %	Cetyl Alcohol %	Isopropyl Myristate %	Propylene Glycol %	Triethnolamine %	Lanoline %	Glycerin %	Nat. most. Agent %	Water Up to %
I	5	20	-	-	10	0.5	-	-	-	64
II	5	10	-	10	10	0.5	-	-	-	64.5
III	5	5	-	10	10	0.5	-	-	-	69.5
IV	5	5	-	10	5	0.5	-	5	-	69.5
V	5	5	2	10	5	0.5	-	5	-	67.5
VI	4	5	10	10	5	0.5	1	5	-	67.5
VII	4	5	10	8	5	0.7	1	5	-	65
VIII	4	5	10	8	5	0.7	0.5	5	-	65.5
IX	4	5	5	8	5	0.7	0.5	5	2	100

Effect of neem leaves extracts on skin renewal:

The dansyl chloride fluorescence method [5] was used to measure skin renewal effect of various dried neem leaves extracts. Dansyl chloride (5-dimethyl-amino-1-naphthlene sulfonyl chloride) is a fluorescent due which stains the skin. It combines avidly with amino group and this is useful for fluorescent tagging of proteins.

a) Preparation of dansyl chloride base:

Dansyl chloride dispersion (5%w/w) was prepared using white soft paraffin as a base, using a glass mortar and pastel. Since dansyl chloride is a light sensitive dye, preparation of the base was carried out in a dark room with subdued red light.

b) Preparation of standard for comparison of fluorescence intensity:

A fresh piece of forearm skin surface of about (10 x 2 cm) was obtained from the Government medical college, Nagpur. It was immediately put in to ice to avoid deterioration and brought to laboratory. Subcutaneous fats adhering to skin were removed with forceps and scissors and the skin was made as clean as possible. The epidermis was then separated by heat-trypsinization method of Klingman [6]. It was cut in to pieces of 1 x 1 cm and stored in a desiccator which was then placed in a refrigerator. Each concentration of dansyl chloride base was liberally and uniformly smeared on the pieces of skin with the help of index finger in dark. The skin pieces were than sandwiched between two glass slides tied with rubber band and preserved in refrigerator below 0OC. This way the slides of different concentration (0.5-5.0% w/w) of dansyl chloride were prepared. After 24 h, the fluorescent intensity of these stained pieces was measured at 340 nm by spectroflurometer. Fluorescence intensity of these standards was checked over a two months period to ascertain that the fluoresces does not decline.

c) Development of fluorescence test patches on human volunteer:

Ten healthy human volunteer, five male and five female, between ages 18–24 years were participated in the study. Informed consent was taken from the participants. Both the inner forearms of each volunteer were used as site for development of patches. On each forearm three marking having dimension of 5 % dansyl chloride base was liberally and uniformly smeared over the marked area on each forearm in dark room with red subdued light. These areas were then covered with occlusive adhesive bandages (Handyplast, India). Volunteers were advised to prevent patches from water contact for at least 24 h. After 24 h the bandages were removed and sites were examined under U.V. radiation to ascertain the development of fluorescence stain.

d) Application of investigational cream on the test patches:

All the dried extracts were dispersed in propylene glycol to prepare 1% w/v. Each volunteer had six patches stained with dansyl chloride, three on each forearm. Out of these six patches; four were treated with investigational cream once a day. The fifth patch was treated with propylene glycol to ensure whether the solvent had any effect on the skin renewal rate or not. The sixth patch was untreated and used as a control. The patches were marked with blue pen by viewing in ultraviolet light chamber. Each extract was uniformly and liberally applied on the respective patch site with rubbing finger to ensure maximum absorption. Volunteer were advised not to apply any cosmetics preparation on these test patches.

Fluorescent intensity of patches were measured by comparing it with standard, prior to application of cream, volunteers feedback report was prepared subsequently which involved observation for skin irritation, if any.

e) Fluorescence intensity determination:

Fluorescence intensity of test patches was measured by keeping forearm in UV radiation chamber, in which patches give fluorescence. The intensity of fluorescence of test patches was compared with that of standard preparation. By visual comparison between standard patches having measured fluorescence intensity and test patches, the test patches were assigned appropriate fluorometric values. These observations were taken on alternate days.

f) Application of varied concentration of prepared cream:

In another set of volunteer study, ten volunteer of either six, participated and six patches were developed on inner aspect of the forearm by method described earlier.

g) Interaction of cream with dansyl chloride stained patches:

To insure that no interaction occurs between dansyl chloride and investigational extracts, 5 % dansyl chloride dispersion was applied on the ten 1 x 1cm patches of stratum cornum prepared by the method of Kligman for twenty four h these were kept in cool and dark place. After twenty four h intensities these patches were determined by comparing with standards in UV radiation chamber. Each prepared cream was applied on two patches and stored in refrigerator. The patches were observed at a definite interval for one month to see whether there is any change in fluorescence or not.

Dansyl chloride staining technique was used for determing the effect of neem leaves extracts on skin renewal rate.

The fluorescence intensities of these patches were measured on alternate days by comparison with standard patches in U V radiation chamber till the fluorescence disappeared completely. Time taken for complete disappearance of fluorescence was noted.

Table 2

Fluorescence intensities of standard dansyl chloride stained slides

Sr. No	Dansyl chloride (%)	Fluorescence intensity (%)
1	5.0	99
2	4.5	91
3	4.0	72
4	3.5	65
5	3.0	52
6	2.5	41
7	2.0	30
8	1.5	22
9	1.0	17
10	0.5	10

The fluorescence intensities were measured at 340 nm by spectrofluorometer. The fluorescence intensities of these standards are tabulated in Table 2, which indicates that fluorescence intensity is directly proportional to concentration of dansyl chloride applied. The decline in fluorescence intensities of the dansyl chloride stained patches due to daily application of cream of different grades (0.5%, 1.0%,and 2.0%) of hydroalcoholic macerate extract of neem leaves are depicted in Table 3.

Table 3
Decline in mean fluorescence intensities with the application of cream containing graded concentration of hydroalcoholic macerate extracts.

Time in days	Control	Hydroalcohol macerate cream (0.5%)	Hydroalcohol macerate cream (1%)	Hydroalcohol macerate cream (2%)
1	91.8	93.6	92.3	91.8
3	71.8	70.8	70.8	67.5
5	56.7	48.3	48.3	46.5
7	42.8	29.6	28.6	27.8
9	27.8	9	7.1	13
12	14.2	2.2	1	
14	10.6			
16	1.5			

The % increase in renewal rate of skin due to topical application of various extracts was calculated by using following formula 1.

$$\%\ \text{increase in renewal} = \frac{\text{Mean No. of renewal} - \text{Mean No. of days for renewal of patch}}{\text{Mean No. of days for removal of control patch}} \times 100 \qquad (1)$$

The percentage increase in renewal rate due to the application of cream containing hydroalcoholic macerate extracts are given in Table 4

Table 4
Percentage increase in renewal rate due to application of cream containing hydro alcoholic macerate extracts

Cream formulation	% increase in renewal rate
Hydroalcohol macerate (0.5%)	23.75 %
Hydroalcohol macerate (1.0%)	26.25 %
Hydroalcohol macerate (2.0%)	27.50 %

Subjective evaluation of the modified cream base:

A panel of 10 volunteers' student was formed. To all the members of panel each formulation was offered. Members were asked to report the formulation, consistency, texture, spreadibility, oclusiveness and washability .the results are as depicted in Table 5.

Table 5 : Successive evaluation of various FAPG modified o/w creams.

Formulation	Consistency	Texture	Spreadibility	washability	Nature	Stability
FAPG base	Poor	Rough	Poor	Good	Oily	stable
I	Poor	Rough	Poor	Good	Oily	stable
II	Poor	Rough	Poor	Good	Oily	stable
III	Poor	Rough	Good	Good	Oily	stable
IV	Good	Smooth	Good	Good	Oily	poor
V	Good	Smooth	Good	Good	Oily	poor
VI	Good	Smooth	Good	Good	Oily	good
VII	Good	Smooth	Good	Good	Slightly oily	good
VIII	Good	Smooth	Good	Good	Non greasy	good
IX	Good	Smooth	Good	Good	Non greasy	good

Stability testing:

Modified cream bases were evaluated for their thermostabiliy .In each case 100 g of cream was taken into two 100 ml beaker. One beaker was kept at room temperature ,while another beaker was kept at $45^{O}C$ for one month .creams were usually observed for any change in consistency, bleeding and phase separation.

Rheological property:

Rheological behavior of the formulation IX was studied by keeping the cream at room temperature ($35^{O}C$), and at elevated temperature ($45^{O}C$) in an oven for one month. The viscosity of the cream were determined by using spindle no 7 at different r.p.m. (1, 5, 10, 50 and100) with the aid of Brookfield viscometer.

Results are depicted in Table 6.

Table 6
Rheological study

Time in days	Viscosity (cps)	
	Room temperature	**45°C**
0	28,000	27,000
2	29,500	26,000
7	31,000	25,000
14	32,000	24,000
22	32,000	24,000
30	32,000	24,000

Viscosity in cps =Dial reading of Brookfield Viscometer x Factor

Result and Discussion

In the FAPG base ,the amount of propylene glycol is very large .propylene glycol acts as as penetration enhancer ,humectant and also exerts solvent action .Excess amount of it causes adverse action on the stratum corneum lipids. It was decided to minimize the amount of propylene glycol without altering its desirable properties. It was also decided to keep 10% of propylene glycol in the formulation by replacing rest of the amount with water.

The preferred emulsifiers for o/w creams are anionic and non ionic type. Non ionic type emulsifiers are temprature sensitive and may pose stability problems in adverse temperature conditions .Therefore it was decided to use Triethnolamine as a emulsifier which is anionic emulsifier and part of it acts with stearic acid to form triethanolamine stearate. This compound acts as an emulsifier for the frmulation of cream. Modified FAPG base formula no 1 reducing propylene glycol with the water and triethanolamine.the cream was found to be very hard and difficult to spread.it was decided to modify the formulation.

The modified formula IX was founed to satisfy in all respect i.e. spreadibility ,consistency ,non greasiness , thermal stability ,washability and texture.

It was decided to use formula IX for the preparaytipon of 0.5% ,1.0% and 2.0% of hydroalcoholic macerate extracts.

The different modified FAPG ceams containing 0.5% ,1.0% and 2.0% w/wof hydroalcoholic macerate were studied on the stratum corneum renewal rate.
six volunteers participated in the study and results shows that hydroalcoholic macerate extracts cream increase skin renewal rate with significant % increase as shown in Table 4.

References

1. Sharma, P.L., Fitoterapia, 1987, 58, 239.
2. Prakash, A. O., Indian Drugs, 1991, 28, 183.
3. Kumar, A., Indian J. Veterinary, 1994, 70(1), 42.
4. Lal, J., Fitoterapia, 1990, 61, 75.
5. Jansen, L. H., Hojyo, M. T. and Klingman, A .M., Br. J. Dermatol., 1974, 90, 9.
6. Baker, H. and Klingman, A. M., Arch .Dermatol , 1967, 95, 40.

*****