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IN VIVO EVALUATION OF SUN LIGHT PROTECTION PROPERTY OF ALOEVERA FORMULATIONS BY DNA FRACTIONATION TECHNIQUE

[1*]V Malarvizhi, [1]C Vijaya, [2]Sudhakar Sekar, [2]Vasudeva Rao

[1]Ultra College of Pharmacy, Madurai, Tamilnadu – 682 711, India.

[2]Faculty of Pharmacy, Asia Metropolitan University, Cheras 43200, Selangor DE, Malaysia.

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***Correspondence for Author**
Dr. V Malarvizhi
Ultra College of Pharmacy, Madurai, Tamilnadu – 682 711, India.

ABSTRACT

Pure *Aloe vera* extract has a mild to severe skin irritant property depending on the type of skin if it is formulated as film forming lotion the chance of skin irritation may be less. In the present study *Aloe vera* sunscreen formulations were prepared by incorporating 10% of *Aloe vera* aqueous extract in film forming lotion and gel. In order to develop a lotion, a film forming polymer (Eudragit RS 100) at 3 levels 10%, 20% and 40% solution in iso propylalcohol with vanishing cream base was used. The consistency and spreadability were checked. Based on the results, Eudragit 10% contained formulation was very suitable for applying on the skin. The *Aloe vera* gel was prepared by using Carbopol 934, Sodium hydroxide, *Aloe vera* extract and Water. In-vivo studies were conducted in Swiss Albino mice to find out the sun protective property of the formulation. The study was carried out for 5 days by exposing the skin to UVB for 15 min in each day. UV light induced DNA damage, evidenced by isolated skin epidermal DNA (genomic DNA) and fractionation of genomic DNA carried out by DNA-agarose gel electrophoresis. DNA fractionation study shows that lotion treated groups of animal genomic DNA was not damaged by UV-B radiation. Furthermore, DNA concentration estimated by UV spectroscopy reveals that lotion treated animal groups epidermal DNA concentration level is more when compared to gel treated groups.

KEYWORDS: Aloevera, Gel, lotion, DNA Fractionation, In-Vivo.

INTRODUCTION

Sunlight, in the broad sense, is the total spectrum of the electromagnetic radiation given off by the sun. [1] Sunlight has three forms of radiations which are infrared [heat / invisible], visible and ultra violet radiation. Ultra violet radiation is classified into three categories [2] viz UV-A, UV-B and UV-C. In that UV-A Light is also known as black light, which causes tanning, UV-B- is 1000 times stronger than UV-A. It is responsible for sunburn and it produce most damaging effects on the skin like reddening or erythema, edema, blistering, aging, wrinkles and cancer. UV-C is filtered out by atmosphere and never reaches us. [2] Sunburn, pigmentation, hyperplasia, immunosuppression, and vitamin D synthesis represent acute responses of the skin to solar UVR, whereas photo aging and photocarcinogenesis constitute chronic effects. Clinical, experimental, and epidemiological evidence associates UV light exposure with development of skin cancer.

Sunscreens can be presented in almost any of the product forms which can be applied to skin so as to give a continuous film, ranging from lotions, aqueous or alcoholic, through liquid and semisolid emulsions products to non-aqueous lipid preparations. Included also are gels and aerosols.*Aloe vera* is a natural sunscreening agents which prevents the skin from UV radiation induced sun burn,[3,4] DNA damage and cancer etc. and also it has a lot of medicinal properties. Additionally *Aloe vera* doesn't have any adverse effect and an easily available plant. There are reports of skin burning following dermal abrasion for removal of acne scars. Rare instance of contact dermatitis (rash) have also been reported [3]. Application of Aloe prior to sun exposure may lead to rash in sun exposed areas. [4]

Pure *Aloe vera* extract has a mild to severe skin irritant property depending on the type of skin [5] if it is formulated as film forming lotion the chance of skin irritation may be less after drying compared to the commercial *Aloe vera* gels and also lotion may have better cosmetic properties. So the present investigation is directed to formulate *Aloe vera* aqueous extract containing film forming lotion and gel and evaluate them for sun protection properties.

MATERIALS AND METHODS

Materials

Aloe vera extract is obtained from Sakthi ayurvedic pharmacy, Madurai, India.Eudragit RS 100 is procured from Roehm Pharma Industry Mumbai, India. Stearic acid and Bromophenol blue purchased from Loba chemie Pvt Ltd (Mumbai, India.). Sodium hydroxide BDH (Qualigens Mumbai, India) and Triethanolamine from Nice Laboratory Ltd (Kochi, India).

Carbopol-934 (Sd Fine Chemicals Ltd (Mumbai, India) Proteinase K (Bangalore Geni, Bangalore, India). Agarose, Phenol and Ethidium bromide (Sisco Research Laboratory Pvt Ltd Mumbai, India). Isopropanol obtained from Qualigens Mumbai, India. Chloroform and Isoamyl alcohol (Merck Specialities Pvt Ltd Mumbai, India).

Animals

Locally breeded female Swiss albino mice of same age group (1 month), weighing 20 ± 2 g, were used and housed under standard environmental conditions (temperature: 27 ± 1°C, light/dark cycle: 10/14 h). The mices were fed with standard pellet diet (Amruth-feed, Bangalore, India) and water *ad libitum*. The institutional animal house is registered with the Govt. of India. Experimental protocols were reviewed and approved by the institutional animal ethics committee.

METHODS

Preparation of *Aloevera* Lotion

A vanishing cream base was prepared in which a film forming polymer would be dispersed in order to form the lotion. The composition of *Aloe vera* lotion is shown in the table 1.

Table-1: Formula for Sunscreen Lotion.

Ingredients	Quantity (%)
Aloe vera extract	10.0
Stearic acid	12.0
Triethanolamine	1.0
Methyl paraben	1.0
Propyl paraben	0.1
Eudragit RS 100 (10%)	10.0
Perfume	Q.S
Distilled water	Upto 100

To prepare vanishing cream base, Stearicacid was melted at 70^{o} C. Aqueous phase ingredients were also heated up to 70^{o} C separately. Then both solutions were mixed well with constant stirring until the cream was formed. Different strength of Eudragit RS100 polymer solutions 10%, 20% and 40% w/v were prepared by dissolving Eudragit RS100 granules in isopropyl alcohol. Then 2 ml of the polymer solution were added to 20 gm of cream base separately so that the strength of polymer in the final formula will be 10%, 20% and 40%w/w and mixed well to get the lotion. The consistency and spreadability of the resulting lotions were compared .The final formulation was prepared by adding 2 ml of *Aloe vera* extract to lotion of desirable quality and mixed well to get the homogenous dispersion.

Preparation of *Aloevera* Gel

Carbomer was dispersed in distilled water and slightly heated to dissolve the carbomer in water. Sodium hydroxide solution was added with slow agitation to prevent inclusion of air. *Aloe vera* extract 2ml was added and mixed well until a homogeneous gel was formed. The composition of *Aloe vera* gel is shown in the table.2.

Table-2: Formula for Sunscreen Gel.

Ingredients	Quantity
Aloe vera extract	10.0
Carbopol 934	0.8%
Sodium hydroxide [10%]	3.2%
Water	86%

***In Vivo* Evaluation of The Sunscreening Effect of The Prepared Formulations on Swiss Albino Mice.**

Swiss albino mice with an initial weight of 20 ± 2 gm were used. A square area of (2.5 x 2.5 cm) on the dorsal side of mouse body was chosen and the hairs were removed. The area was cleaned using a cotton swab dipped in water before applying sunscreens. The animals were divided in to five groups. Group 1 and group 2 animals were treated topically with *Aloe vera* lotion and gel in the concentration of 2 mg/cm^2 respectively. They were spread as uniformly as possible with the finger and allowed to remain on the skin for 15 minutes before irradiation. Group 5 was treated with pure *Aloe vera* extract and group 4 was not treated topically, but irradiated. Group 3 was served as control. Then the animals were placed in leather cuff with a hole of 2.5 x 2.5 cm size punched in it, allowed the UV-B radiation to reach only this area. For UV-B exposure groups of animals were immobilized under a thin wire net of 14 x 14 cm at a distance from the UV- B lamp (O Panel 00, UV-B 313). The study was carried out for 5 days by UVB exposing the skin for 15 min in each day. At the end of 5 th day, the animal skin biopsies were taken and used it for further study.

Isolation of Genomic DNA From Uv-B Radiation Induced Skin Tissue Of Mice [6]

Immediately after excision, the tissue is washed with saline H_2O to free from blood and minced it quickly in eight volume of ice cold homogenizing buffer. The buffer is previously prepared by the formula shown in table 3. Then it's filtered through three layers of cheese cloth and centrifuged the filtrate at 4^{o}C for 10 minutes at 3000 rpm in Cooling centrifuge (Remi, C24)

Table-3: Formula for homogenization Buffer.

Materials	g /100 ml
Sucrose	8.557
NaCl	0.073
KCl	0.093
$MgCl_2$	0.106
Tris-Cl pH 7.5	0.788

The supernatant liquid is discarded and suspended the nuclear pellet in desired volume (20 µl) of suspension buffer prepared as per table 4. Then the nuclear pellet volume is adjusted with 50 µl of 10% SDS (sodium dodecyl sulphate) and 50 µl of 0.7 M NaCl (50:50) and centrifuged at 3000 rpm for 10 minutes. Again the supernatant liquid is removed and 20 µl of proteinase K to a final concentration of 100 µg/ml is added and incubated the sample at 50^{o}C for 1 hour. Thoroughly the sample is extracted with an equal volume of 150 µl of phenol/ $CHCl_3$/ isoamyl alcohol in the ratio (25:24:1) and centrifuged at 3000 rpm for 10 minutes. Thick layer of white sponge solution was formed and repeated the organic extraction. The aqueous layer is transferred to another tube and added 0.5 vol (5µl) of 7.5 M ammonium acetate solution and 2 vol (20µl) of 100% ethanol. Then the DNA is recovered by centrifugation at 3000 rpm for 10 minutes. The recovered pellets were rinsed with 70% ethanol and decant the ethanol or air dried the pellets. Re-suspend the DNA in Tris Cl- EDTA buffer (TE) until it is dissolved DNA may be solubilized by incubating at 65^{o}C for several hours. TE buffer is prepared by the formula shown in table 5.

Table- 4: Formula for Suspension Buffer.

Materials	mg / 10 ml
NaCl	5.84
$MgCl_2$	6.09
Tris-Cl pH 7.5	15.76

Table- 5: formula for TE buffer.

Materials	g / 100 ml
Tris-Cl pH 7.5	0.121
EDTA	0.0375

Quantification of DNA to determine the purity and concentration [7]

Dilute 0.2µl aliquot of DNA solution with 2 ml of milli Q water.Transfer the solution to a quartz cuvette. Milli Q as reference and absorbance at 260 and 280 nm was measured by using UV-double beam spectrophotometer (Systronics) and results were noted in table 6. From that, concentration and purity of DNA sample was determined and the results were noted in table 7.

Table- 6: Uv Absorbance of Skin Dna of Swiss Albino Mice.

Sample	Absorbance	
	260nm	280nm
Normal	0.539	0.905
Untreated	0.322	0.493
With gel treated	0.40	0.686
With lotion treated	0.470	0.801
With extract treated	0.389	0.669

Dna Fractionation By Agarose Gel Electrophoresis [7]

Casting and Running Gel Electrophoresis

1.2% Agarose gel is prepared by using 0.36g agarose, 3 ml of 10X buffer and Ethidium bromide 0.5µg/ml. The agarose was boiled to dissolve by placing the flask in boiling water bath for about 15 min.The agarose was completely dissolved and the flask was removed from the water bath and left at room temperature to cool. After that ethidium bromide solution (10µl) is added. The platform (8 x 11 cm) of Horizontal gel tank with power pack (Apelex, cat no: 311000, minicel2 sim 110203) was washed with distilled water and wiped dry with tissue paper and open ends were sealed securely with cellophane tape. The comb was placed 1 cm from the top end and make sure that the teeth of the comb do not touch the surface of the platform .The platform was placed on a smooth horizontal surface. Once the agarose solution is cooled to about 50^0 C, then the solution is poured gently to cover the entire surface of the plate form and left undisturbed for about 30mins. 15 ml tris boric acid – EDTA buffer made it to 150 ml with distilled water is used as running buffer.

15 µl of DNA sample mixed with 5 µl of loading dye (bromo phenol blue) and the sample loaded in to the well by using pipettman. The electrophoresis is started by switched on the D.C powerpack. The bromophenol blue have moved from bottom end, after 30 – 45 min, the gel was rinsed with Distilled water and by keeping platform in a slanting position, the gel was gently pushed on to the UV transilluminator. DNA bands were seen and photographed using an orange filter of Gel documentation system (Vilber Lourmat, CE V03 7833)

RESULTS AND DISCUSSION

Preparation of Sun Screen Film Forming Lotion

The present study was undertaken to evaluate sun protection efficacy of *Aloe vera* in a film forming lotion formulation, comparatively to that of the commercial type gel. Sunscreen formulated with stearic acid and triethanolamine produced thick cream. It was easy to apply and left an indiscernible film on the user's skin [6]. Hence the formula for the sunscreen base

includes stearic acid and triethanolamine. Literature survey reveals to use alkyl poly vinyl pyrrolidones such as copolymers of polyvinyl pyrrolidone with eicosene or dodecene, acrylic resins, methacrylic resin and mixtures there of as a polymer. One of the water insoluble acrylic resins Eudragit RS 100 was selected for film formation [8].

Water insoluble film forming polymers are added to semisolid sunscreen composition to provide matrix or binder which physically envelops the sunscreen with the aid of the oil-soluble solubilizer, preventing the sunscreen from being absorbed by the skin .The polymers also prevent the sunscreen from being rubbed off by physical contact with clothing, towels, furniture, other parts of the body (e.g. scratching the protected area) etc [8].

Estimation of DNA By Uv-Absorbance Method

Five groups of sample absorbance were noted and the values are given in table 6. It is based on the fact that conjugated bonds in nitrogenous bases of nucleic acids absorb light strongly at 260 nm based on the amount of UV light absorbance at 260 nm the nucleic acid concentration was calculated as follows:

The concentration of DNA was calculated by using formula.

DNA (g/ml) = A 260 X 50 X Dilution factor ---------- (1)

A 260 = unit of single strand nucleicacid (DNA)

A 260 = unit of duplex nucleicacid

50 = extinction co-efficient

The DNA concentration of each group was given in table 7. The concentration of DNA in lotion treated groups was higher than that of other formulation and comparable to that of normal group DNA. These results clearly indicate that UVB damages DNA and lotion prevents these UVB induced DNA damage thereby protecting the skin from harmful effects of UVB radiations.

Table- 7: Determination of DNA Concentration.

Sample	DNA Concentration (g/ml) at 260nm
Normal	0.02695
Untreated	0.0161
With gel treated	0.02
With lotion treated	0.0235
With extract treated	0.01945

DNA Fractionation

Epidermal DNA from irradiated and different sunscreen protected irradiated mouse skin epidermal DNA was fractionated by agarose gel electrophoresis.[10,11] Five experimental conditions (untreated, gel treated, lotion treated, extract treated, normal) were tested. DNA isolation from epidermal was carried out to evaluate the DNA fraction in agarose gel electrophoresis. The analysis of DNA Fragment mobility distribution showed (fig. 1) that much smaller DNA fragments were found in the untreated mouse skin compared with sunscreen protected mouse skin.Experimental animals have correlated sunscreen efficiency with prevention of DNA damage[9]. They demonstrated that *Aloe vera* lotion could significantly reduce DNA damage from the DNA fractionation in gel electrophoresis showed that sunscreen (*Aloe vera*) provided an efficient block against UVR.

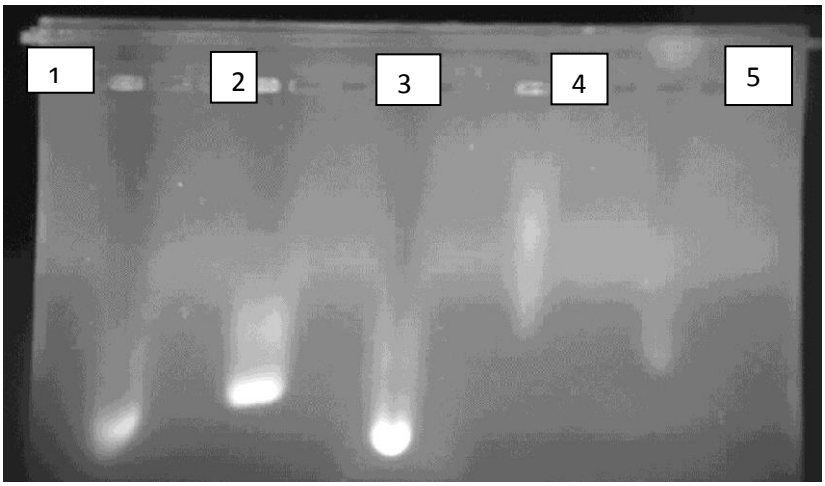

Fig-1: DNA fractionation by agarose gel electrophoresis.

Lane 1 – Lotion treated group; Lane 2 – Gel treated group; Lane 3 – Normal group; Lane 4 – untreated group; Lane 5- Extract treated group.

CONCLUSION

Aloe vera film forming lotion is prepared successfully using Eudragit RS 100 film forming agent. DNA estimation reports that lotion treated animal groups epidermal DNA concentration level is more when compared to other treated groups. DNA fractionation studies show that lotion treated groups of animal genomic DNA was not damaged by UV-B radiation because the length of lane was nearly about the normal group lane. Pure *Aloe vera* extract has a mild to severe skin irritant properties depending on the type of skin. If it is formulated as a film forming lotion the chance of skin irritation may be less after drying compared to the *Aloe vera* gel.

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