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Research Article

Remediation of Ultraviolet-Rays Induced Skin Damages by the Phytochemical Composition of Herbal Extracts

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ABSTRACT

Background & Objectives: Plant derived drugs came into use in the modern medicine through the uses of plant material as indigenous cure in folklore or traditional systems of medicine. The present study report the remediation of UV-A and UV-B rays induced sun burn by the phytocombination of different medicinal plant extracts in the form of herbal mixture. Material & Method: The current study was designed to elucidate the effect of the formulated herbal mixture at the dose of 100mg/kg bwt. against skin alterations induced by ultraviolet radiations at the web length of 290-400nm *i.e.* UVA and UVB for 5minuts up to 4 weeks on *Swiss albino* mice. pH test of herbal mixture and Histological observation of the skin of treated animals were also undertaken. Results: Single topical applications of herbal mixture at the dose of 100mg/kg bwt have shown significant reduction in skin wrinkles and inflammation which was induced by continuous exposure of ultraviolet radiations in *Swiss albino* mice. The results were interpreted in grade manner. The histopathological observations of skin of animals also support the protective potential of this herbal mixture which was recovered damaged Keratinocytes, Collagen, Elastic fibers etc. Conclusion: The present investigation must be an important key for the therapeutic drug discovery process for the photoprotection by the nature with no side effects. These results may help in the formation of herbal sunscreen lotion with low side effects and without using synthetic chemical substances.

Keywords: Herbal mixture; Photoprotection; Phytochemical; Skin damages; Ultraviolet radiations.

INTRODUCTION

Sunburn or Erythema is redness of the skin, which is due to increased blood flow in the skin caused by dilatation of the superficial blood vessels in the dermis as a result of exposure to UV radiation. Sunburn is an acute cutaneous inflammatory reaction that follows excessive exposure of the skin to ultraviolet radiation (UVR). Ultraviolet radiations are divided into three categories according to the wavelength: UV-A(400-315nm), UV-B(315-280nm) and UV-C(<280nm). Repeated exposure of UV-B results in an aged skin (Photoaging)[1]. UVR exposure can come from a variety of sources, including sun, tanning beds, phototherapy lamps, and arc lamps[2]. Everyone's skin and eyes can be affected by the sun and other forms of ultraviolet (UV) rays. People with light skin are much more likely to have sun damage, but darker-skinned people, including people of any ethnicity, can also be affected. High UV doses may also results in edema, pain, blistering, and peeling of the skin a few days following exposure. The skin is composed of three main layers, the uppermost epidermis, the lower dermis and the hypodermis. Each of these layers is composed of a specific set of cells that perform an essential function in that layer. The epidermis contains keratinocytes, melanocytes and langerhans cells which are critical for the structural and functional integrity of the epidermis. Keratinocytes are the major population of cells and originate in the bottom most stem cell pool in the stratum spinosum. Chronic exposure of mammalian skin to UV radiation induces a number of biological responses, including development of erythema, edema, sunburn cell formation, hyperplasia, immune suppression, DNA damage, photoaging and melanogenesis. These alterations are directly or indirectly involved in the development of skin cancer[3-5]. Exposure to UVR also increases the incidence and/or severity of infections in mice challenged with bacterial, fungal, viral, or parasitic agents[6] and therefore has important implications for susceptibility to infectious diseases and vaccine effectiveness in humans. UVR suppresses cell-mediated immune (Th1) responses, leaving humoral (Th2) responses intact[7]. UV exposure to the skin results in generation of reactive oxygen species. ROS are constantly generated in keratinocytes and fibroblasts, and are rapidly removed by nonenzymic and enzymic antioxidant substances. These prevent harmful effects of ROS and maintain a pro-oxidant/antioxidant balance, thus resulting in cell and tissue stabilization. ROS comprise a number of active metabolites including hydroxyl radical, superoxide anion and peroxyl radical and their active precursors namely singlet oxygen, hydrogen peroxide and ozone. Nitric oxide and nitric dioxide, reactive nitrogen species (RNS), are also generated. One approach to protecting humans from the harmful effects of UV irradiation is to use active photoprotectives. In recent years, naturally occurring compounds have gained considerable attention as protective agents. Vitamins C, E, and β-carotene have

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been incorporated into many skin care products for instance. Another approach is afforded by the antioxidative properties of phenolics. These substances can be used as ingredients in human diet or added to preparations for topical application[8]. There is an immense need to explore the sunburn protective properties of herbal plants because they are rich source of phytoconstituents and antioxidants. The present research deals with the investigation of remediation of photodamages which was induced by UV radiations (UV-A & UV-B) by the phytochemical combination of herbal extracts in the form of herbal mixture using *in vivo* protocol.

MATERIAL & METHOD

Collection and Identification of herbal plants

Fresh plant parts were collected from Bhopal and forests of the Hoshangabad (M.P.) having rich diversity of medicinal plants by the permission of forest authorities. The Plant parts were identified by the Botanist at the department of Botany & Biotechnology, Sadhu Vaswani College, Bairagarh, Bhopal. Plant material was washed thoroughly and shade dried at room temperature. The material was crushed using mortar- pestle and grinding machine. Powders were stored at room temperature in airtight containers.

Reagents and chemicals

One commercially available sunscreen lotion (SPF 20) was purchased from local market of Bhopal for the standard. Ethanol (Merck®) and other chemicals were analytical grade and purchased from CDH, Renchem, Hi-Media Ltd., India.

Extraction of herbal plants

The powder of *Berberis aristata* roots was subjected to 50% ethanolic extraction by soxhlet apparatus at 60^0C temperature and tagged as DH1. The bark powder of *Ficus benghalensis* was extracted with 30% ethanol at 80^0C by soxhlet apparatus and tagged as FB1. Coarsely powdered *Asparagus racemosus* root were extracted with ethanol and water. The part one extracted with ethanol by Maceration, tagged as ST1 and other part was extracted with water by percolation method, tagged as ST2. The flower powder of *Butea monosperma* was extracted with 30% methanol using a soxhlet unit at 70^0C temperature and tagged as PSE1. Fresh leaves of *Aloe vera* were collected, washed with tap water, peeled and gel was collected with the help of spatula in a beaker. Collected gel was dried at 40^0C in incubator. The dried sample was weighed and tagged as AV1. The bark powder of *Terminalia arjuna* was subjected for soxhlet extraction at 80^0C temperature using 80% ethanol as the solvent and labelled as AJ1. 80% ethanolic extract of *Cyperus rotundus* root powder was prepared by hot extraction process using soxhlet unit at 80^0C temperature and tagged as Ngm1. The 80% ethanolic extract of *Rubia cordifolia* root powder was prepared using soxhlet unit at 80^0C temperature and tagged as M2. The flower powder of *Hibiscus-rosa-sinensis* was extracted with 50% methanol by maceration process at room temperature and tagged as Cr2. Collected residues were kept at 45^0C in water bath to concentrate it and finally transfer into hot air oven to dry it. Dried extracts were collected, weighed and kept into air tight containers for further use.

Preparation of herbal mixture

Herbal mixture was prepared by different plant extracts at different Concentrations. The herbal extracts and their quantities were selected on the basis of their reported therapeutic properties such as; demulscent, emollient, aphrodisiac, brain tonic, astringent, febrifuge, aphrodisiac, purgative, antidiarrheal, antidysenteric, cardiotonic and effective on skin diseases[9] and phytochemical analysis and inhibitory concentration values. Each herbal extract was previously screened for phytochemical analysis and antioxidant potential using standard methods of Harborne, 1973[10] and Halliwell *et al.,* 1987[11]. The present phytochemicals and IC_{50} values of each extract were observed and only final standardized data has been summarized in Table-1. Total of 10% herbal mixture was prepared which was dissolved in 50% ethanol. It is reported that maximum of 50% ethanol could be used in cosmetic preparation[12]. Hence solubility of extracts was detected taking 10% to 50% of ethanol in DDW. The maximum solubility was observed in hydroalcoholic (50ethanol:50DDW) solution.

Animals

Experimental animals were handled according to the Institutional Legislation, regulated by the Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. Random bred of *Swiss albino* mice (7- 8 weeks old, both sexes), weighing 23 ± 2 gm body wt. obtained from the animal colony of Research Centre were used for the experiments. These animals were housed in polypropylene cages in the animal house at temperatures of 22 ± 1.5^0C and light (12L: 12D). The animals were provided with standard pallet diet (from Golden feed Ltd., New Delhi, India) and water *ad libitum.* One day before the commencement of the experiment, hairs on the interscapular region of the mice were removed using hair removing cream.

pH testing of herbal mixture

pH were tested for the herbal formulation which was prepared by the phytochemical combination of different extracts of medicinal plants. pH describes the alkaline ratio of a substance which ranges from 0 (most acidic)-14(most alkaline). The normal skin pH is understood to be the pH values of a healthy person. The mean values lay in the ranges 5.4-5.9. This parameter mainly depends on the area of skin and on age.

In vivo sun burn protection study

Experimental design

This experiment was performed as per the method followed by Sachdeva and Katyal, (2011)[13] with required modifications. The animals were randomly divided into 5 different groups and each group comprised of four animals. Hairs were removed with the help of hair removing cream from the dorsal region with proper care in the area of $2cm^2$ in all the groups. The animals were exposed in UV Radiation at the web length of 290-400nm *i.e.* UVA and UVB for 5minuts up to 4 weeks. Following treatment groups were considered; Untreated Group (No

Table 1: Composition of various plant extracts

S. No.	Plant extracts	Quantity (in mg)	Phytochemical analysis	Antioxidant potential (IC_{50} Value)
1.	Hydroalcoholic extract of *Berberis aristata* root (DH1)	5mg	Carbohydrates, Alkaloids, Tannins, Terpenoids, Flavonoids	67.8 µg/ ml
2.	30% ethanolic extract of *Ficus benghalensis* bark (FB1)	4mg	Carbohydrates, Phenol, Tannins, Terpenoids	48.00 µg/ ml
3.	Ethanolic extract of *Asparagus racemosus* root (ST1)	17mg	Carbohydrates, Phenol, Tannins, Terpenoids, Glycosides	38.00 µg/ ml
4.	Aqueous extract of *Asparagus racemosus* root (ST2)	12mg	Carbohydrates, Phenol, Tannins, Terpenoids	45.50 µg/ ml
5.	30% methanolic extract of *Butea monosperma* flowers (PSE1)	2mg	Carbohydrates, Tannins, Glycosides	17.60 µg/ ml
6.	Gel extract of *Aloe vera* (AV1)	25mg	Carbohydrates, Tannins, Phenol	34.50 µg/ ml
7.	80% alcoholic extract of *Terminalia arjuna* bark (Aj1)	20mg	Alkaloids, Glycosides, Tannins, Phenol, Terpenoids, Saponins	37.80 µg/ ml
8.	80% ethanolic extract of *Cyperus rotundus* root (Ngm1)	5mg	Carbohydrates, Alkaloids, Glycosides, Tannin, Phenol, Flavonoids, Saponin	87.00 µg/ ml
9.	80% ethanolic extract of *Rubia cordifolia* root (M2)	6mg	Carbohydrates, Alkaloids, Tannin, Phenol, Flavonoids	50.00 µg/ ml
10.	Hydroalcoholic extract of *Hibiscus-rosa-sinensis* flowers (Cr2)	4mg	Carbohydrates, Glycosides, Tannin, Phenol	58.00 µg/ ml

Table 2: Grading scale for evaluation of photo-damaging

Grade	Evaluation criteria
0	No wrinkles or laxity; fine striations running the length of the body
1	Fine striations
2	Disappearance of fine striations
3	Shallow wrinkles
4	A few deep wrinkles and laxity
5	Increases deep wrinkle
6	Severe wrinkles; Development of tumors/lesions

treatment), Vehicle Control Group (100µl hydroalcoholic solution was given at once a day up to 4 weeks) and Three Experimental Groups (UVR exposed group: Animals were exposed in UVR (UVA+UVB) for 5minuts, once a day upto 4 weeks), (Standard sunscreen treated group: 100µl of marketed sunscreen (SPF20) was applied at the dose of 4mg/kg bwt 1 hour before the exposure of UVR (UVA+UVB) for 5min once a day upto 4 weeks) and (Herbal mixture treatment group: 100µl of herbal mixture at the dose of 100mg/kg bwt was applied on the skin 1 hour before the exposure of UVR (UVA+UVB) for 5min once a day upto 4 weeks). The route of administration was topical.

Visual evaluation of dorsal skin

The skin of treated mice examined for photodamages at the end of 4th week. Under anesthesia, the UV exposed dorsal skin of each mouse to be photograph. The grading scale ranges from 0 for normal skin to 6 for severity photo damaged skin.

Statistical Analysis

Table 3: Depicting pH values of formulated herbal mixture at different concentrations

S. No.	Concentrations of formulated herbal mixture	pH Values
1.	0.5%	4.2
2.	1%	4.8
3.	5%	5.0
4.	10%	5.6

The differences of the sun burn among different groups were considered to be significant at 5% significance level ($p<0.05$), when evaluated by Student's 't' test.

Histological Observations

For histopathological study, a portion of Skin tissue from each animal was removed after dissection and preserved in 10% formalin.

RESULTS

pH Study of herbal mixture

The potential hydrogen (pH) was tested for the herbal mixture which was prepared through the mixing of different plant extract at different quantities in 50% ethanol based on the solubility. Total of 10% herbal mixture was prepared for the study having the pH approximate 3.8-4. The different concentrations were prepared from this stock Viz., 0.5%, 1%, 5% and 10%. These concentrations of herbal formulation have shown pH values as 4.2, 4.8, 5.0 and 5.6 respectively. The normal skin pH is 4-6. The pH of an ideal sunscreen lotion should be near about skin's pH. Results are summarized in table no.3.

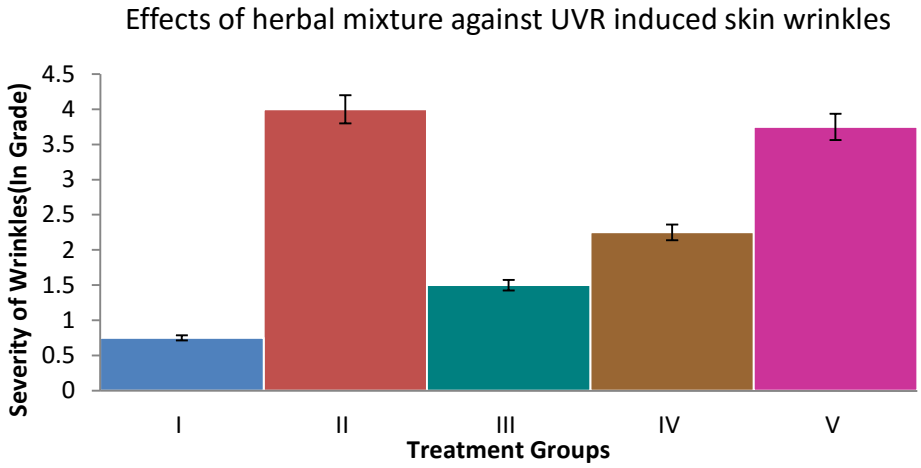
In vivo sun burn protection study

Ultraviolet radiation causes damage to the skin, which may result in both precancerous and cancerous skin lesions and acceleration of skin ageing. Topical administration of herbal formulations which contains secondary metabolites

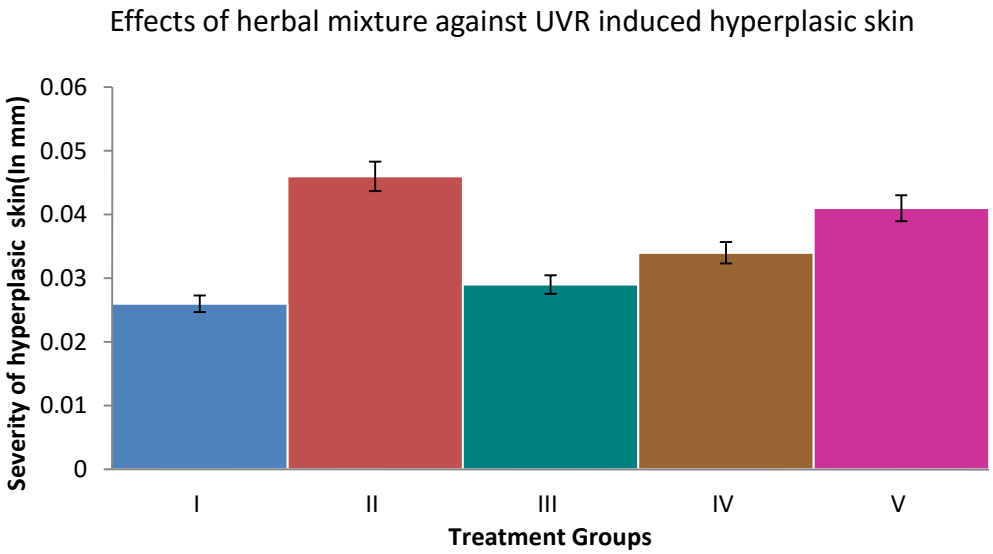
Table 4: Representing effects of formulated herbal mixture against UVR (UVA+UVB) induced Photodamages in *Swiss albino* mice

S. No.	Treatment gp.	Treatment Doses	Route of administration	UVR Induced Photodamages (In Mean±SD)			
				Effect on Body weight		Severity of Wrinkles (In Grade)	Severity of hyperplasic skin (In mm)
				Initial	Final		
1.	I (n=4)	Normal mice	-	26.7±1.6	30.3±1.9	0.75±0.55	0.026±0.001
2.	II (n=4)	UVR exposed (UVA+UVB)	-	23.5±0.20	28.9±0.5	4.00±0.67	0.046±0.012
3.	III (n=4)	Standard (Marketed sunscreen SPF20 4mg/kg bwt.)	Topical	27.35±0.39	28.9±0.5	1.5±0.91	0.029±0.001
4.	IV (n=4)	Experimental (Formulated herbal mixture100mg/kg bwt.)	Topical	26.0±1.4	25.0±1.3	2.25±1.59*	0.034±0.002*
5.	V (n=4)	Vehicle control	Topical	25.6±2.2	28.8±2.8	3.75±0.92	0.041±0.002

*denotes statistical significance as compared to UVR exposed group at $p<0.05$ followed by Student 't' test.

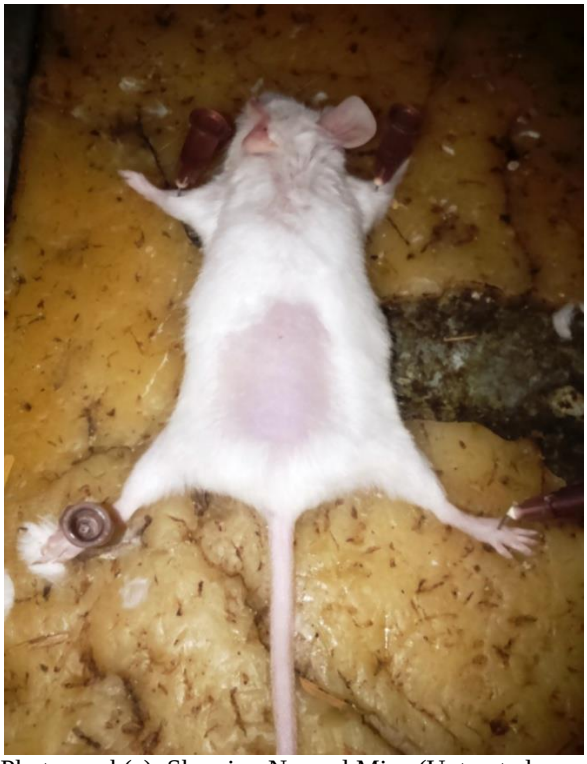


Graph-I: Showing effects of herbal mixture against UVR (UVA+UVB) induced Skin wrinkles up to 4 weeks

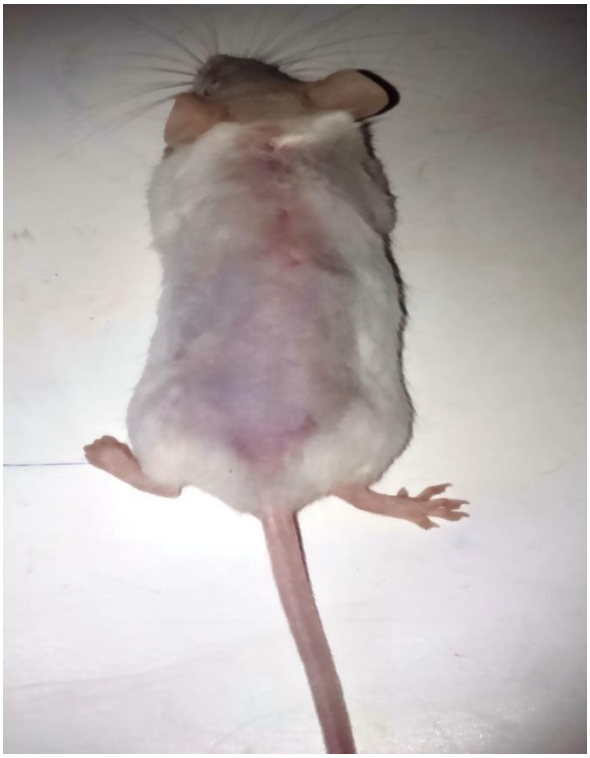


Graph-II: Showing effects of herbal mixture against UVR (UVA+UVB) induced Skin hyperplasic up to 4 weeks

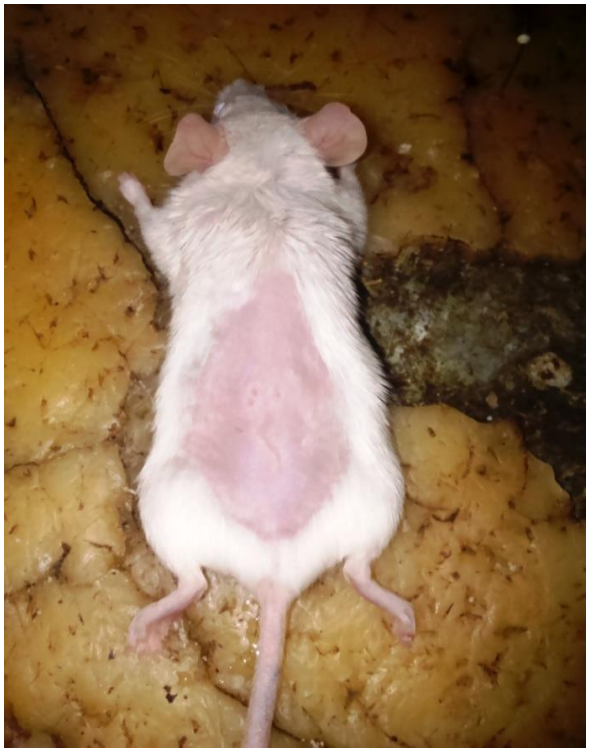
and antioxidants is an effective strategy for protecting the skin against UVR damages. The present investigation revealed that single topical application of herbal mixture at the dose of 100mg/kg body weight 1 hour before the exposure of UVA & UVB for 5minuts up to 4 weeks have shown significant protection against UV-damages with comparison of marketed sunscreen treated group. The effects were studied on the Body weight, Severity of



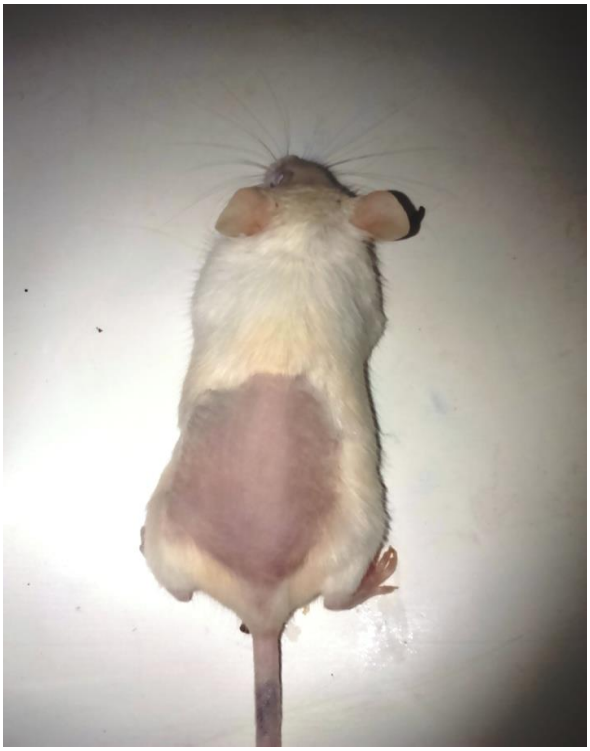
Photograph(a): Showing Normal Mice (Untreated group)



Photograph(b): Showing UVR(UVA+UVB) exposed group upto 5minuts

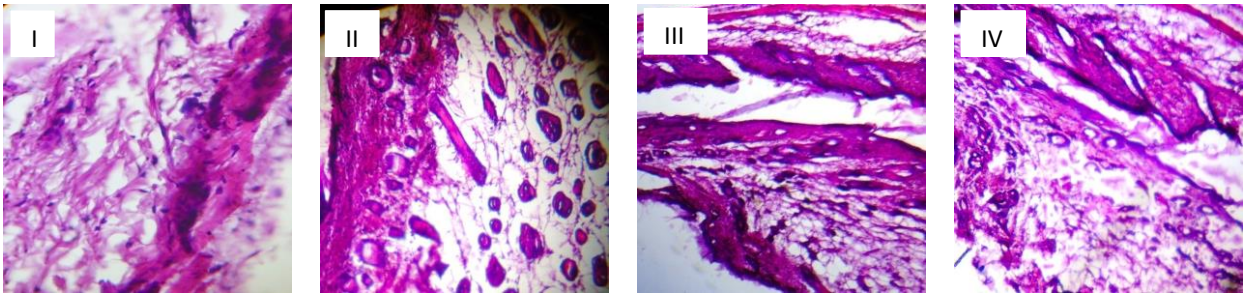


Photograph(c): Showing marketed sunscreen treated group 1hour prior to UVR exposure



Photograph(d): Showing herbal mixture treated group 1 hour prior to UVR exposure

Wrinkles and Severity of Thickened skin (hyperplasic skin). Wrinkles were studied in Grade manner in which 4.00±0.67 grade were observed in UVR exposed group which shows A few deep wrinkles and laxity on skin of animals. Thickness was found to be increased up to 0.046±0.012mm in UVR exposed group when compared with Normal mice (0.026±0.001mm). The experimental group which were receiving topical application of herbal mixture have shown reduced Wrinkles at 2.25±1.59 grade which shows approximate Disappearance of fine striations



Microphotographs: Histological section study of skin of animals**: (I)** Showing histological section of untreated skin of animals **(II)** Showing histological section of UVR induced Photodamaged skin of animals **(III)** Showing histological section of marketed sunscreen treated skin of animals **(IV)** Showing histological section of herbal mixture treated skin of animals

as similar standard group which expressed wrinkles at 1.5±0.91 grade when compared with UVR exposed group. The severity of thickness of skin was found to be reduced up to 0.034±0.002 mm in herbal mixture treated group and standard group expressed 0.029±0.001 mm when compared with UVR exposed group. Vehicle control group, which is an hydroalcoholic solution treated group, have also shown increased thickness of skin at 0.041±0.002 mm and wrinkles were 3.75±0.92 grade which express Shallow wrinkles and few deep wrinkles and laxity on skin of animals. Results were found to be statistically significant in Student 't' test at $p<0.05$ levels. No significant effect was observed on the body weight in all the groups. Results are summarized in Table 4, graphs I, II and photographs (a,b,c,d).

Histological observations

Results of histological studies provided supportive evidence for photoprotective potential of herbal mixture. The Fibroblasts, Collagen, Elastic fibers and no. of Langerhans cells were found to be reduced, epidermal cells get flattened and keratinocytes became altered in the form of sun burnt cells (keratinocytes were found with increased pyknotic nucleus and eosinophilic cytoplasm) in UVR exposed group when compared with the normal untreated group while the group which were receiving standard marketed sunscreen lotion revert all the skin damages to quite normal. The herbal mixture treated group showed the prominent effects on photodamaged skin when applied topically compared with the UVR exposed group and marketed sunscreen treated group. The Microphotographs (I, II, III, IV) are arranged above as supportive evidence. It seems that, the herbal mixture was effective on photodamaged skin and this formulation may be included in the formation of good quality of sunscreen lotion and anti-burning ointments.

DISCUSSION

Healthy human skin is an important physical barrier to the environment and protects the body from a variety of insults. It is the largest human organ and comprises approximately 15 percent of a person's body weight and covers about 1.5 to 2.0m^2 of our surface area. Skin is mainly composed of water (70%), protein (25%) and lipids (2%) forming an effective barrier to dehydration, pathogens and mechanical insults such as abrasion. In a mechanical sense, the main function of the skin is to serve as a protective barrier to keep good things in (water, nutrients and heat) and keep bad things out (pathogens, UV radiation, toxins)[14]. UV has many effects on skin physiology, with some consequences occurring acutely and others in a delayed manner. One of the most obvious acute effects of UV on the skin is the induction of inflammation. UVB induces a cascade of cytokines, vasoactive and neuroactive mediators in the skin that together result in an inflammatory response and causes sunburn. If the dose of UV exceeds a threshold damage response, keratinocytes activate apoptotic pathways and die. Such apoptotic keratinocytes can be identified by their pyknotic nuclei and are known as sunburn cells. UV also leads to an increase in epidermal thickness, termed hyperkeratosis. By causing cell injury, UV induces damage response pathways in keratinocytes. Since UV-induced DNA mutations represent a major causative factor for melanoma and other skin cancers, it follows that resistance to UV-mediated mutagenesis is a critical determinant of skin cancer risk[15]. Ultraviolet radiation is a potent inducer of superoxide radical ($^{+}O_2$), hydrogen peroxide (H_2O_2) and hydroxy radical (^{+}OH), which have been implicated in cutaneous aging including benign and malignant tumors, and various inflammatory disorders[16]. The skin is equipped with a network of protective antioxidants. They include enzymatic antioxidants such as glutathione peroxidase, superoxide dismutase and catalase, and nonenzymatic low-molecular-weight antioxidants such as vitamin E isoforms, vitamin C, glutathione (GSH), uric acid, and ubiquinol. UVR exposure affects the skin antioxidants. Ascorbate, GSH, SOD, catalase, and ubiquinol are depleted in UV-B exposed skin, both dermis and epidermis. Dietary antioxidants thus play a major role in maintaining the homeostasis of the oxidative balance. Vitamin C (ascorbic acid), vitamin E (α-tocopherol), beta-carotene, and other micronutrients such as carotenoids, polyphenols, and selenium have been evaluated as antioxidant constituents in the human diet[17]. Medicinal plants are the versatile source of phytochemicals such as; Polyphenols, Flavonoids, Alkaloids, Saponins, Terpenoids, Tannins, Glycosides etc. and many antioxidant molecules. There are lots of medicinal plants available in nature those have been studied for their photoprotective values and their products are commercially available in market as cosmetics. In this list of research *Aloe Vera* is a prominent example of medicinal

plant which is commonly used in cosmetics, sunscreen lotions and anti-burning ointments because of its adorable medicinal value due to presence of phytotherapeutic molecules and antioxidants. Natural products that are used to treat the symptoms associated with photoaging and photo damage are designed to either stimulate new skin cell formation or inhibit the biochemical processes that cause skin damage. Botanical extracts are the most popular sources of naturally sourced bioactives in skin health clinics. Most often, these are mixed into topical creams and applied to the epidermis. Flavonoids are polyphenolic compounds typically found in plants. These compounds are ideal candidates for cosmetic purposes because of their potent bio-activities and low toxicity. There are many reports of flavonoids from various sources interfering with or reducing the activity of tyrosinase, and although they all show some efficacy at reducing the biochemical pathways that lead to pigmentation[14]. The present research revealed photoprotective effects of an herbal mixture which was formulated by composition of different medicinal plant extracts such as hydroalcoholic extract of *Berberis aristata* root, 30% ethanolic extract of *Ficus benghalensis* bark, alcoholic extract of *Asparagus recemosus* root, aqueous extract of *Asparagus recemosus* root, 30% methanolic extract of *Butea monosperma* flowers, gel extract of *Aloe vera*, 80% alcoholic extract of *Terminalia arjuna* bark, 80% ethanolic extract of *Cyperus rotundus* root, 80% ethanolic extract of *Rubia cordifolia* root and hydroalcoholic extract of *Hibiscus-rosa-sinensis* flowers in an hydroalcoholic solution. Further we have assessed the effects of herbal mixture against UVR (UVA+UVB) induced photodamages such as; skin wrinkles and thickened skin in *Swiss albino* mice and results were compared with marketed sunscreen treated animals. Topical applications of herbal mixture against UVR induced skin damages in animals have shown significant reduction in skin wrinkles and inflammations. The histopathological observation of skin of animals also support the protective potential of this herbal mixture in which an improvement was observed in damaged Keratinocytes, Collagen, Elastic fibers. The Antioxidant activity is important in UV protection[18]. This herbal formulation has potential to protect the skin from harmful ultraviolet radiations may be due to its antioxidant activity and presence of phytoconstituents. There is an immense need of more investigation and characterization of active principle responsible for its photoprotective activity.

CONCLUSION

In ancient Indian literature, it is mentioned that every plant on this earth is useful for human beings, animals and other plants. Present research work has confirmed the potential ethno pharmacologically active natural compounds having photoprotective activity and exhibited strong antioxidant potential which is helpful in skin care and preparation of cosmetic in Industries. This research may give active, safe and causally-acting plant derived preparations that will be able to replace some synthetic chemical substance in the formation of sunscreen lotion. The bioactive compounds of this herbal mixture may able to reduce skin damages which caused due to long time exposure of skin in sun rays specially UVA and UVB rays. These results may help in the formation of herbal sunscreen lotion with low side effects and without using synthetic chemical substances. This research may give future aspects in the field of cosmetic industries for the formation of antiburning ointments and sun protective creams.

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