International Journal of Medicine and Pharmaceutical Research
CODEN (USA): IJCPNH | ISSN: 2321-2624
Journal Home Page: www.pharmaresearchlibrary.com/ijmpr

Evaluation of Antipsoriatic activity from *Azadirachta indica* leaves by Ethanolic extract in Wistar rats

Yalamuri Chandravadana*, Karamala Swetha

Department of Pharmacology. Krishnateja Pharmacy College, Chadalawada Nagar, Tirupathi-517506, Chittoor (DT), A.P.

A B S T R A C T

Psoriasis is a chronic autoimmune and noncommunicable inflammatory disease of skin and joints. The disease has a worldwide prevalence of two percent, with a higher prevalence of about 4.6% in developed countries. It is characterized by having sharply demarcated scaly, red, coin-sized skin lesions most often on the elbows, knees, scalp, hands and feet. Number of herbal plants were evaluated for anti-psoriatic activity and documented. Traditionally, *Azadirachta indica* leaves were used for various skin diseases and especially for psoriasis. Mouse tail test and UV radiation induced psoriasis were used for the evaluation of antipsoriatic activity. Extracts were tested at a dose of 100 mg/kg b.w. and fractions at 200 mg/kg b.w. in Wistar rats. The present study supports the use of ethanolic extract of Azadirachta indica at dose level of 200 mg/kg for psoriasis treatment.

Keywords: Psoriasis, UV -Ray, *Azadirachta indica,* ethanolic extract.

A R T I C L E I N F O

***Corresponding Author**
Yalamuri Chandravadana
Department of Pharmacology,
Krishnateja Pharmacy College,
Chadalawada Nagar, Tirupathi-517506, Chittoor (DT), A.P.

A R T I C L E H I S T O R Y: Received 05 December 2020, **Accepted** 31 Jan 2021, **Available Online** 10 February 2021

Citation: *Yalamuri Chandravadana`, et al.* Evaluation of Antipsoriatic activity from *Azadirachta indica* leaves by Ethanolic extract in Wistar rats. *Int. J. Med. Pharm. Res., 2021, 9(1): 18-27.*

CONTENTS

1. Introduction

Psoriasis is a chronic autoimmune and no communicable inflammatory disease of skin and joints. The word psoriasis comes from a Greek word “Psora” which means being itchy and “iasis” means a condition. The disease has a worldwide prevalence of two percent, with a higher prevalence of about 4.6% in developed countries. It is characterized by having sharply demarcated scaly, red, coin-sized skin lesions most often on the elbows, knees, scalp, hands and feet. Symptoms include itching, irritation, stinging and pain. Rarely, the entire skin surface of the body may be involved. Neem (*Azadirachta indica*) plants parts shows

antimicrobial role through inhibitory effect on microbial growth/potentiality of cell wall breakdown.

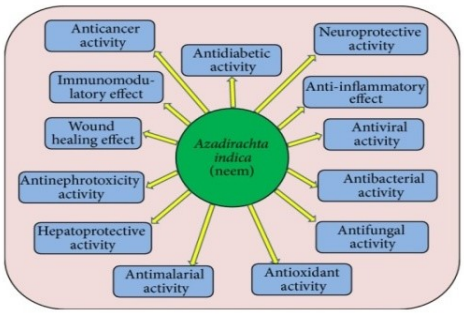


Figure 1: Pharmacological activities of *Azadirachta indica*

Plant-*Azadirachta indica*

Botanical Classification

Kingdom - Plantae
Order - Sapindales
Family - Meliaceae
Genus - *Azadirachta A.Juss.*
Species - *Azadirachta indica A.Juss.*

Plant description

Azadirachta indica tree belongs to the family Meliaceae which is found in abundance in tropical and semitropical regions like India, Bangladesh, Pakistan, and Nepal. It is a fast-growing tree with 20–23 m tall and trunk is straight and has a diameter around 4-5 ft. The leaves are compound, imparipinnate, with each comprising 5–15 leaflets. Its fruits are green drupes which turn golden yellow on ripening in the months of June–August.

Chemical Constituents

Azadirachta indica shows therapeutics role in health management due to rich source of various types of ingredients. The most important active constituent is azadirachtin and the others are nimbolinin, nimbin, nimbidin, nimbidol, sodium nimbinate, gedunin, salannin, and quercetin. Leaves contain ingredients such as nimbin, nimbanene, 6-desacetylnimbinene, nimbandiol, nimbolide, ascorbic acid, n-hexacosanol and amino acid, nimbiol 17 hydroxyazadiradone, 7-desacetyl-7-benzoylazadiradione, 7-desacetyl-7-benzoylgedunin. Quercetin, ß-sitosterol, polyphenolic flavonoids, were purified from neem fresh leaves and were known to have antibacterial and antifungal properties and seeds hold valuable constituents including gedunin and azadirachtin.

Figure 2: Images showing flowers, fruits and leaves of *Azadirachta indica*

2. Material and Methods

Materials

Collection of plant material

The plant material *Azadirachta indica*was collected from Tirupathiand its surrounding areasand authenticated by an expert plant taxonomist Dr. K. Madhava Chetty (IAAT : 357).

Experimental Animals:

Healthy Wister rats of either sex aged between 2-3 months and weighing 150–200 g were used for the study which was procured from Teena Bio labs Pvt. Ltd. (Reg. no. 177/99 CPCSEA), Hyderabad, Telangana. The animals were housed in Krishna Teja Pharmacy college in standard polypropylene cages, and maintained under standard conditions (12:12 hour light and dark cycle; at an ambient temperature of 25 $\pm$ 5^{o}C; 35-60% of relative humidity). The animals were fed with standard rat pellet diet and water *ad libitum*. The maintenance and the handling of animals were done according to Committee for the Purpose of Control and Supervision on Experimentation on Animals (CPCSEA) guidelines. The antipsoriatic activity of the ethanol extract was investigated using ultraviolet B(UV-B)-induced photodermatitis model in rats. The animals were divided into four groups (6 rats/groups). The vehicle-control group animals received normal saline (10 ml/kg, p.o.) and standard group received retinoic acid (0.5 mg/kg, p.o.). Remaining groups were treated orally with the ethanolic extract of leaves of *Azadirachta indica*(200 and 400 mg/kg, p.o.) and data were analyzed using one-way analysis of variance (ANOVA).

Ethical Approval:

All the pharmacological investigations were carried out only after obtaining Institutional Animal Ethical Committee (IAEC) approval.

Chemicals

Retinoic Acid was obtained from Manus Aktteva Biopharma LLP, Ahmedabad, and Gujarat. Ethanol, gum acacia, hydrochloric acid, chloroform etc.

Instruments: UV- Visible spectrophotometer- (UV- B), Soxhlate Apparatus, Rotary Evaporator, Analytical balance.

Preparation of the Ethanolic Extract of *Azadirachta indica:* The leaves of plant material were shade dried at room temperature. The dried material was then crushed by mechanical grinding and stored in a dry place until use. The coarsely powered whole plant material was subjected to soxhalation using ethanol in 60:40 ratios for 72 hrs, at 60-80°C.The concentrated extracts were obtained by evaporating the solvent, under reduced pressure in a rotary evaporator at 42-45^{o} C.The concentrated extracts were transferred to china dishes and then dried at room temperature. The solid extracts were scraped before complete drying, and then dried to a constant weight. The percentage yield obtained was 16.05% w/w and kept in an air tight container until use. The dried *Azadirachta indica* ethanolic extract was suspended in 2% gum acacia and used for the present study.

Preliminary Phytochemical Screening of Ethanolic Extract

A. Detection of Glycosides: About 50mg of extract was hydrolyzed with concentrated hydrochloric acid for two hours on a water bath, filtered and the hydrolysate was subjected to the following tests.

Borntrager's test:

To 2ml of filtered hydrolysate 3ml of chloroform was added and was shaken, chloroform layer was separated and 10% ammonia solution was added to it. Pink colour indicates presence of glycoside.

Legal's test:

About 50mg of the extract was dissolved in pyridine. Sodium nitroprusside solution was added and made alkaline by using 10% sodium hydroxide solution. Presence of glycoside is indicated by pink colour.

Keller – Killiani Test:

To a small quantity of the crude ethanolic extract acetic acid and a drop of ferric chloride solution and to it concentrated Sulphuric acid was added along the sides of test tube. A brown ring was observed at the junction of two layers indicating the presence of de-oxy sugars.

B. Detection of Flavonoids

Magnesium and HCL Reduction Test:

The crude ethanolic extract was dissolved in a few ml of alcohol and few pieces of Magnesium ribbons and concentrated Hydro Chloric acid was added drop by drop. Pink or crimson red colour developed indicating the presence of flavonoids. The extract and fractions showed positive response for the above test.

C**. Test for Phenolic compounds:**

Ferric Chloride Test:

To the ethanolic extract Ferric chloride solution was added. The appearance of blue colour indicates the presence of Phenolic compounds.

D. Test for Steroids/Terpenoids:

Liebermann-Burchard Test:

The extract was dissolved in acetic anhydride by heating the mixture to boiling, cooled and then 1ml of cold concentrated sulfuric acid was added along the sides of the test tube. Color change at the junction was observed. Steroids/Triterpenoids and their glycosides give red, pink or blue color.

E. Test for Alkaloids:

About 50mg of the extract was stirred with few ml of dilute Hydrochloric acid and filtered. The filtrate was subjected to various alkaloid reagents. The details of the tests are given hereunder.

Mayer's test:

To a few ml of filtrate, 2-3 drops of Mayer's reagent was added along the sides of the test tube. A creamy precipitate indicates presence of alkaloids.

Wagner's test:

To a few ml of filtrate, 2-3 drops of Wagner's reagent was added along the sides of the test tube. A reddish-brown precipitate indicates presence of alkaloids.

Hager's test:

To a few ml of filtrate, 2-3 drops of Hager's reagent was added along the sides of the test tube. A yellow precipitate indicates presence of alkaloids.

Dragendroff's test:

To a few ml of filtrate, 2-3 drops of Dragendroff's reagent was added along the sides of the test tube. A reddish-brown precipitate indicates presence of alkaloids.

E. Test for Carbohydrates:

Molisch Test: To the extract taken in a test tube, 1ml of water and 1ml of 5% (w/v) alcoholic α-naphtol solution were added and mixed well. The mixture was cooled and 1ml of concentrated sulfuric acid was added along the sides of the test tube. No color developed at the junction was recorded. Carbohydrates give a violet color at the junction.

F. Test for Saponins:

Froth Test: A small quantity of the extract was taken into a test tube. To it, was added 15ml of water and shaken vigorously and set aside. The froth produced was observed after 15 minutes. Saponins, if present, produce a stable froth.

H. Detection of proteins and amino acids:

About 100mg of extract was dissolved in 10ml of distilled water and filtered through Whatman No.1 filter paper and the filtrate was subjected to tests for proteins and amino acids.

Million's Test:

To 2ml of filtrate, few drops of Million's reagent was added. A white precipitate indicates the presences of proteins.

Biuret Test: An aliquot of 2ml of filtrate was treated with one drop of 2% copper sulphate solution to this 1ml of (95%) ethanol was added, followed by excess of Potassium hydroxide pellets. Pink color in the ethanolic layer indicates presence of proteins.

Ninhydrin Test: About 2 drops of Ninhydrin solution was added to two ml of aqueous filtrate. A characteristic purple color indicates presence of amino acids.The extract and fractions shows negative result for the above tests.

Acute Oral Toxicity Study (OECD. 2001)

Acute Toxicity study for the ethanolic extract of *Azadirachta indica* was carried out on mice according to OECD guidelines. Three mice were fasted overnight and maintained with water *ad libitum*. Each animal received single dose of ethanolic extract of *Azadirachta indica* (2000 mg/kg, p.o). After administration of the test compounds, animals were observed individually and continuously for 30 min, 2 hr and 24 hr to detect changes in the autonomic or behavioral responses and also for tremors, convulsion, salivation, diarrhea, lethargy, sleep and coma and then monitored for any mortality for the following 15 days. No mortality or any other autonomic or behavioral responses such as tremors, convulsion, salivation, diarrhea, lethargy, sleep and/or coma were observed during first 15 days.

Rat UV ray photo dermatitis model for psoriasis on Wistar rats: Irradiation of the depilated Wistar rat skin was carried out with UV radiation. Immediately after irradiation, initial faint erythema appears that last for 30 min. The second phase of erythema starts after 6 h and gradually increases, peaking between 24 and 48 h. This reaction is confined to the exposed area and has a sharp boundary, and develops a brownish-red color. By 48 to 72h, silvery white scale appears on the erythematous lesion. These scales are relatively thick and begin to fall beyond 72 h. Although the erythematic reaction is induced artificially, many of the pathological features resemble those seen in psoriasis vulgaris. The close resemblance of inflammatory process produced by UV radiation to the one exhibited in psoriasis provides a good model to investigate drugs that have a potential to reduce the inflammatory reaction associated with psoriasis.

Procedure

The hairs of the Wistar rat skin, on one side of the flank, were depilated by clipping with a scissors followed by careful shaving taking precaution to avoid injury to the skin. The rats were then placed on a curved wooden block and their legs tied around it, to avoid contact with the floor. This arrangement prevented the movement of the animal during its subsequent exposure UV radiation. Except for an area of 1.5 × 2.5 cm on the depilated skin, the entire animal was covered with a UV-resistant film. The uncovered area of 1.5 × 2.5 cm was then irradiated for 20 min with a UV-B lamp kept at a vertical distance of 20 cm from the skin. The rats were divided into four groups (6 rats/groups). The vehicle-control group rats received normal saline (10 ml/kg, p.o.) and standard group received retinoic acid (0.5 mg/kg, p.o.). Remaining groups were treated orally with the ethanolic extract of leaves of Azadirachta indica (100 and 200 mg/kg BW) once daily, 5 times a week, 12 h after irradiation for 2 weeks. Two hours after the last treatment, animals were sacrificed under ether anesthesia; longitudinal sections of the tail skin were made and prepared for histological examination with hematoxylin-eosin staining.

Histopathological Examination

Sections were examined for presence of Munro's microabscesss, elongation of rete ridges, and capillary loop dilation by direct microscopy. The vertical epidermal thickness between the dermoepidermal junction and the lowest part of the stratum corneum (n = 3 measurements per scale, n = 3 scales per animal, n = 6) were examined. The percentage relative epidermal thickness of all the groups was calculated in comparison with the positive control group (100%; n = 54 measurements per treatment). It was also examined for mean thickness of stratum corneum and stratum granulosum.

Statistical Analysis:

All results are expressed as mean ±S.E.M (standard error of mean). Statistical evaluation was done using one way analysis of variance (ANOVA), followed by Dunnett's method. Statistical calculations were done and the graphs were prepared using Graph pad prism version 5.0

Evaluation for Anti-Psoriaticactivity

Perry Scientific Mouse tail Model:

This is accepted as a screening method for measuring anti psoriatic activity of *Azadirachta indica*. The basis of this method is that topical treatment of a mouse-tail with anti-psoriatic drugs enhances orthokeratotic cell differentiation in the epidermal scales. This characteristic is utilized for direct measurement of drug efficacy in an animal model. Drugs are applied topically, once daily, 5 times in a week, for 2 weeks. As an indicator of orthokeratosis, the number of scale regions with a continuous granular layer is counted and expressed as a percentage of total number of scale regions per section. Drug activity is defined by the increase in percentage of orthokeratotic regions.

Extracts tested:

The successive ethanolic isolated compounds of *Azadirachta indica* leaves were screened for anti psoriatic activity. Each extract (100 mg) & isolated compounds (50 mg) were formulated in the form of a cream, using liquid paraffin (10 ml) and bees wax (3 gm) and appliedtopically.

Standard used:

Retino-A 0.05% (Tretinoin cream U.S.P.) - Janssen-Cilag Pharmaceuticals (Trademark of Johnson & Johnson, U.S.A.) in cream form was used as a standard.

Induction of Psoriasis:

All groups of animals were exposed to UV light for inducing psoriasis on skin.

Procedure:

Male Wistar rats weigh in garound 300g are used. Proximalendoftail, an area on one side of the flank is irradiated for 20 min (1.5 J/cm2) at a vertical distance of 20cm with UV lamps. Abiphasicerythemais observed. Immediately after irradiation, initial faint erythema appears, disappearing within 30 min. The second phase of erythema starts 6 h after the irradiation and gradually increases, peaking between 24 & 48h. The color is brownish-red, and the reaction is confined to the exposed area with asharpboundary. By 48-72 h after irradiation, dark brown scale is formed on the erythematous lesion. Pieces of the scale are relatively thick.

Method of Screening:

Screening of ethanolic extracts is carried out with reference to the standard. Extracts we are applied topically once daily, 5 times a week, for the period of 2 weeks. Drugs are applied topically, once daily, 5 times in a week, for period of 2 weeks. Two hours after the last treatment animals were sacrificed; longitudinal sections of the tail skin were made and prepared for histological examination (hematoxylin eosin staining). As an indicator of orthokeratosis the number of scale regions with continuous granular layer is counted and expressed as a percentage of the total number of scale regions per section. Drug activity is defined by the increase in percentage of orthokeratotic regions.

Antipsoriatic Activity – Mouse Tail Test

Azadirachta indica leaves extract was evaluated for antipsoriatic activity by the mouse tail test for psoriasis. Twenty four animals were divided into 4 groups of six each. Group I served as normal control (0.1% Normal saline), group II served as reference control (Retino-A, 0.05%) and group III was treated with the 100mg/kg body weight of *Azadirachta indica* leaves of ethanolic extract, group IV was treated with 200mg/kg body weight of Azadirachta indica leaves of ethanolic extract. Test drugs were administered once daily for 14 days, by suspending in 0.1 % normal saline solution. At the end of the 14th day treatment, mice were sacrificed by phenobarbitone anesthesia and the proximal parts of their tails were cut and each group tails stored in separate containers containing 10 % formalin in saline.

Histopathological Examination

Longitudinal histological sections were prepared from the tail skin and stained with hematoxylin eosin. The specimens were histometrically analyzed for:

(I) The horizontal length of an individual scale lying in between adjacent hair follicles including sebaceous glands (n = 10 scales per animal, n= 6 animals per treatment group; i.e. a total of 60 measurements per treatment),

(II) The horizontal length of the fully developed granular layer within an individual scale (n = 10 scales per animal, n = 6 animals per treatment group; i.e. a total of 60 measurements per treatment), and

(III) The vertical epidermal thickness between the dermo-epidermal junction and the lowest part of the stratum corneum (n = 5 measurements per scale, n = 10 scales per animal, n = 6 animals per treatment group; i.e. a total of 300 measurements per treatment). From these raw data (I to IV) the following parameters were calculated.

(IV) The degree of orthokeratosis of an individual scale defined as the percentage ratio of (2) divided by (1) (n = 60 data per treatment condition),

(V) The control related 'drug activity' upon epidermal differentiation,

$$\text{Drug activity} = \frac{\text{OKs} - \text{OKc}}{100 - \text{OKc}} \times 100$$

Where, OK-orthokeratosis

Orthokeratosis as the mean of the parameter explained under (IV) for a test substance (s) and the untreated control condition (c), respectively, and (IV) the relative epidermal thickness of individual scales as the percentage ratio of the measure under (III), for a given treatment in relation to the mean of untreated controls set to 100% (n = 300 data per treatment condition). The three overall parameters namely, the degree of orthokeratosis, drug activity and relative epidermal thickness were eventually used for the evaluation of drug effects.

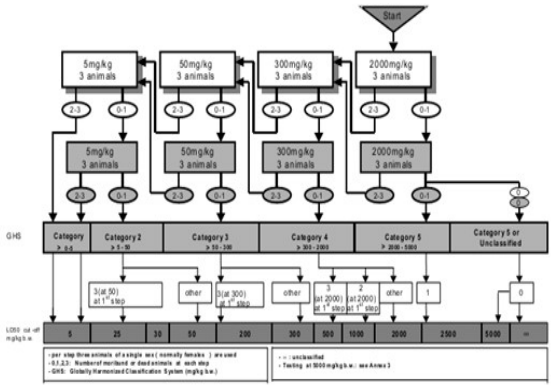


Figure 1: Pharmacological Investigations

Table 1 Animals were divided into groups as follows

Group (n=6)	Treatment
Group-I	Vehicle Control (Normal saline 10 ml/kg, p.o.)
Group-II	Retinoic acid (0.5 mg/kg, p.o.) Standard group.
Group-III	100mg/kg body weight of *Azadirachta indica* leaves ethanolic extract.
Group-IV	200mg/kg body weight of *Azadirachta indica* leaves ethanolic extract.

3. Results and Discussion

Phytochemical Investigations: The various qualitative chemical tests performed on the test extract showed that the extract contained glycosides, flavonoids, and phenolic compounds, steroids, terpenoids, alkaloids and saponins.

Acute Toxicity Test: The acute toxicity test was performed on the mice and no abnormality or mortality was seen with 2000 mg/kg dose of test extract given orally. Hence the test dose was fixed as 100 and 200 mg/kg. [25] Wistar rats were divided into three groups of six rats each. A limit test at a dose of 2000 mg/kg body weight was carried out of ethanol extract. The animals were observed for clinical signs and mortality for 15 days. Body weight was recorded every week. The tested sample was found to be safe and did not produce any mortality after 15 days. The acute toxicity studies, the ethanolic extract were administered orally and animals were observed for mortality and behavioral responses. No mortality was observed in rats treated with 2000 mg/kg of ethanolic extract. All the rats were normal and no gross behavioral changes were observed till the end of the study period. Acute dermal toxicity Healthy young adult animals of commonly used laboratory strains were employed. Each animal was 8–12

weeks old which was selected and all animals' weights were within the range. The animals are acclimatized to the laboratory conditions for 5 days before the start of the study. Animals are randomly selected to use in the study and marked to provide individual identification. Approximately 24 h before the study, fur was being removed from the dorsal area of the trunk of the test animals by shaving. The test substance was applied uniformly over an area which is approximately 10% of the total body surface area. Test substances were held in contact with the skin with a help of porous gauze dressing and nonirritating tape throughout a 24 h exposure period. Animals were observed for signs of toxicity from 30 min to periodically during the first 24 h, with special attention given during the first 4 h. All animals were free from signs of toxicity.

Pharmacological Investigations

Rat UV-ray Photo Dermatitis Model for Psoriasis

Histopathological examination revealed fully developed Munro's microabscess, elongation of rete ridges, and capillary loop dilation. The mean thickness of the epidermis, mean thickness of the stratum corneum and mean thickness of the stratum granulosum in control and drug treated animals were tabulated. There was significant decrease in epidermal thickness ($P < 0.05$) as compared with control group. The section of vehicle-control group showed regular elongation of rete ridges, capillary loop dilation with minimal grade lesion of diagnostic Munro's microabscess and marked increase in relative epidermal thickness as compared with other groups. In ethanol extract (100 mg/kg) treated group, there was a minimal grade lesion of elongation of rete ridges along with capillary loop dilation in the section and absence of Munro's microabscess. In ethanol extract (200 mg/kg) treated group, there was no lesion of Munro's microabscess, capillary loop dilation along with elongation of rete ridges in the section of skin of rats. In the standard group, there was absence of Munro's microabscess, capillary loop dilation along with elongation of rete ridges in the section showing significant therapeutic effects when compared with test-treated groups.

Evaluation of anti-psoriatic activity of test compounds

Epidermal thickness of mice tail was determined to observe the severity of psoriasis, physical observation of animal skin including erythema, itching and slivery patches on tail region. Psoriasis score index was calculated for each group to compare the efficacy of test compounds with that of standard. Psoriasis area and severity index (PASI) scores were determined by evaluating the degree of erythema, thickening and scaling on the affected dorsal skin surface and ear pinna. PASI for each was measured on a 4-point scale (0 = none; 1 = slight; 2 = moderate; 3 = marked and 4 = very marked). The severity of skin inflammation was measured by the combined scores (erythema plus scaling plus thickening) giving a range of scores of 0–12. Histological parameters are also observed by collecting the tail skin of mice.

Psoriasis Severity Index: Psoriasis Severity Index was gave to all individual group animals based on their signs and presence of erythema, itching and silvery scales on the skin. Vehicle control group gain score of 2.0 at the 1st week, it was increased to 2.4 at the end of the experiment (3rd week), standard group gain 2.7, at the 1st week, after that application of drug on the topically to the skin, reduction in signs and symptoms of psoriasis slowly and 52% reduction is seen at 3rd week that 1.0. Test treated groups all were initially gained the score 2.0 to 2.8 at the 1st week, it was slowly reduced to the 2nd week; totally (55–70%) reduction in PSI is observed at the time of the 3rd week of treatment period (Table 8).

Table 2: Showing Phytochemical screening

Constituents	Observation
Reducing sugars	-
Alkaloids	-
Flavonoids	+
Saponins	-
Tannins	-
Sterols/terpene	+

(+) indicates presence of chemical constituents
(-) indicates absence of chemical constituents

Table 3: Effect of test compounds on Psoriasis Severity Index

Group	1st week	2nd week	3rd week
Vehicle control (Disease control)	2.0	2.4	2.7
Standard (Retino-A, 0.05%)	2.1	1.3***	1.0***
Ethanolic extract of *Azadirachta indica* (100mg/kg)	2.3	1.5***	1.3***
Ethanolic extract of *Azadirachta indica* (200mg/kg)	2.8	1.8***	0.9***

Table 4: Histopathological features on UV-B-induced psoriasis in Wistar rats treated with ethanolic extract of *Azadirachta indica*

Group no=6	Treatment	*Histopathological Examination*					
		Thickness of stratum corneum	Thickness of stratum granulosum	Percentage of relative epidermal	Munro's microabscess	Elongation of rete ridges	Capillary loop dilation

		(µm)	(µm)	thickness (µm)			
I	Vehicle Control (Normal saline 10 ml/kg, p.o.)	2.85±0.75	0.35±0.1	93.49±3.17	++	+++	++
II	Standard (Retenoic acid 0.5mg/kg, p.o)	17.83±2.01	11.96±1.81	23.02±1.49***	-		
III	Ethanolic extract of *Azadirachta indica* (100mg/kg)	7.44±0.41	4.36±0.91	48.74±2.49 ***	-	+	+
IV	Ethanolic extract of *Azadirachta indica* (200mg/kg)	10.96±1.35	6.33±1.29	42.14±2.82***	-	-	-

+=Mild grade lesion, ++=Moderate grade lesion, +++=Severe grade lesion, -=No lesion, n=6; V. Data were analyzed by one-way ANOVA followed by Dunnett's method. *P<0.05, **P<0.01, *** P<0.01, when compared against vehicle control group.

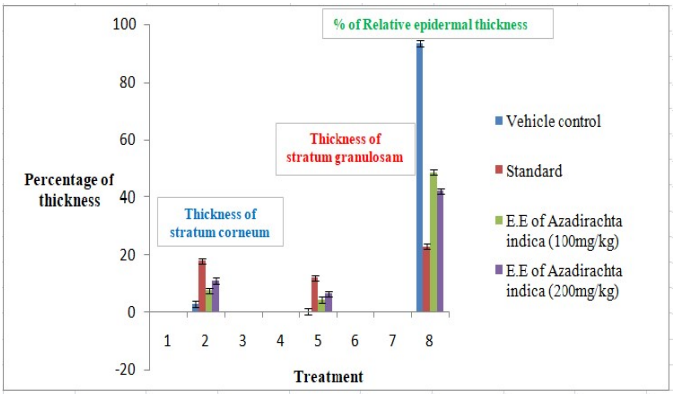
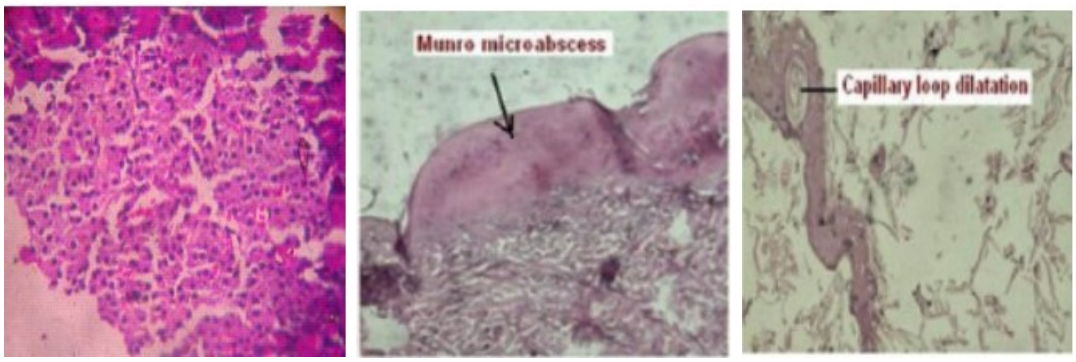


Figure 3: Histopathological studies psoriasis of Azadirachta indica leaves of ethanolic extract in Wistar rats

The mean thicknesses of the epidermis, in control- and ethanolic extract-treated animals, were tabulated above table (Table no 3). There was significant decrease in epidermal thickness ($P < 0.05$) as compared with vehicle (disease control) control group. The section of disease control group showed regular elongation of rete ridges, capillary loop dilation with minimal grade lesion of diagnostic Munro's microabscess and marked increase in relative epidermal thickness as compared with other groups. In ethanolic extract of *Azadirachta indica* (100mg/kg) of treated group, there was a minimal grade lesion of elongation of rete ridges along with capillary loop dilation in the section and absence of Munro's microabscess. In ethanolic extract of *Azadirachta indica* (200mg/kg) treated group, there was no lesion of Munro's microabscess, capillary loop dilation along with elongation of rete ridges in the section of skin of rats. In the standard group, there was absence of Munro's microabscess, capillary loop dilation along with elongation of rete ridges in the section showing significant therapeutic effects when compared with test treated groups.



A) Control B) Munro's microabscess C) capillary loop dilation

Figure 4: Histopathological studies of UV B induced psoriasis treated groups

Table 5: Effect of *Azadirachta indica* leaves extract on the degree of orthokeratosis and relative epidermal thickness in the mouse tail test

Group (n=6)	Drug Treatment	Degree of orthokeratosis	Drug activity (%)	Relative epidermal thickness (%)
I	Vehicle Control (Normal saline 10 ml/kg, p.o.)	19.15±0.92	-	98.52±5.45
II	Standard (Retenoic acid 0.5mg/kg, p.o)	75.15±2.45***	68.78	134.38±4.65
III	Ethanolic extract of *Azadirachta indica* leaves (100mg/kg)	32.25±5.12***	29.52	58.32±5.15
IV	Ethanolic extract of *Azadirachta indica* leaves (200mg/kg)	70.91±3.67***	63.81	123.62±4.94

Values are expressed as mean ± S.E.M, *P<0.05, **P<0.01, *** P<0.01 Vs control group

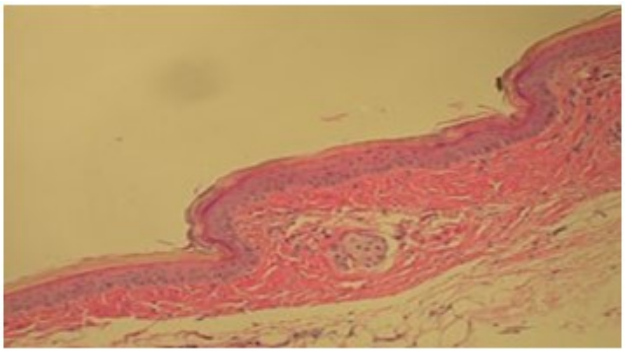


Figure 6: Shows an untreated control which reveal sorthokeratotic regions

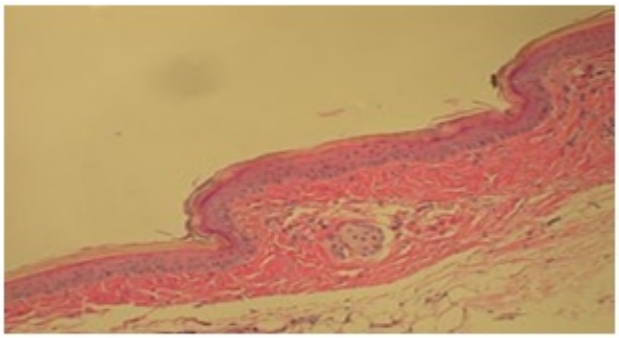


Figure 7: Shows treatment with Retin A, a commercially available ointment with anti psoriasis activity.

Discussion

Skin is the largest exposed organ of body and easy target for allergic and immunologic reactions. Skin ailments viz. dermatitis, urticaria, angioedema, psoriasis, etc., are immune mediated chronic, inflammatory disorder. Psoriasis severely affects patients with the quality of life and the treatment being expens ive. Medicinal plants are considered safe, as for the human health and are widely employed by the traditional healers for the treatment of various diseases including psoriasis. Medicinal plants are known to be a rich citadel of variety of chemical compounds and have attracted researcher's attention to find new treatment for psoriasis .Screening of antipsoriatic activity of ethanol extract of Azadirachta indica leaves was carried out using UV-B-induced psoriasis in rat. The irradiated rat skin treated with ethanol extract of Azadirachta indica leaves (200 mg/kg) has shown a significant reduction in the total epidermal thickness indicating that it has an influence to retard the hyper proliferation of the keratinocytes that occurs when the skin is exposed to UV radiation. The significant retention of the stratum granulosum is probably due to its ability to enhance the keratinization process, which is a protective strategy adopted by the skin when exposed to penetrating radiation. Further, ethanol extract of Azadirachta indica leaves (200 mg/kg) produced useful changes in the epidermis of the irradiated skin, showing its potential use. Anti-psoriatic activity of the leaves extract of Azadirachta indicaleaveswas evaluated in the mouse tail test method, the % Degree of Orthokeratosis and the % Relative Epidermal Thickness were studied and the result were given in table 10. Keratotic condition is seen in the adult mouse tail which is one of the main features of psoriasis. Induction of orthokeratosis in the adult mouse tail is the basis behind the mouse tail test. Retinoic acid was used as reference drug and ethanolic leaves extract of Azadirachta indica leaves of ethanolic extract (100 mg/kg) and (200 mg/kg) were used in the study. Retinoic acid and the test drug Azadirachta indicasignificantly (P>0.001) enhanced the degree of Orthokeratosis and Relative Epidermal Thickness in the mouse tail method. The % drug activity of retinoic acid and Azadirachta indicaleaves of ethanolic extracts of test dose concentrations of (100 mg/kg and 200 mg/kg) were 68.78%, 29.52%and 63.81% respectively.

4. CONCLUSION

Psoriasis is a chronic and disabling disease which affects the quality of life. Number of herbal plants were evaluated for anti-psoriatic activity and documented. Traditionally, *Azadirachta indica* leaves were used for various skin diseases and especially for psoriasis. Present study was conducted to evaluate the anti-psoriatic effect of *Azadirachta indica* ethanolic extract on mouse tail test method. From the result it was concluded that ethanolic leaf extract of *Azadirachta indica* exhibited.From the above study we can conclude that leaves of *Azadirachta indica* ethanolic extract at doses 100mg/kg and 200mg/kg studied. In that ethanolic extract of Azadirachta indica at

dose level of 200mg/kg exhibited significant activity on UV-induced psoriasis in Wistar rats. The study implies that selected plant is a promising research for further development to prove its anti psoriatic activity.

Acknowledgement

Authors sincere thanks to the management and principal of Krishan Teja Pharmacy college, for their support to do the research work.

5. Bibliography

1. Aldeen T, Basra M. Management of psoriasis and its comorbidities in primary care. Br J Nurs. 2011, 20:1186, 1188–90, 1192.
2. Ritchlin, Christopher; Fitzgerald, Oliver. Psoriatic and Reactive Arthritis: A Companion to Rheumatology (1st ed.). Maryland Heights, Miss: Mosby; 2007. p.4. ISBN 978-0-323-03622.
3. Lowes MA, Bowcock AM, Krueger JG. Pathogenesis and therapy of psoriasis. Nature. 2007; 445:866–73.
4. Cannavò SP, Riso G, Casciaro M, Di Salvo E, Gangemi S. Oxidative stress involvement in psoriasis:a systematic review. Free Radic Res. 2019; 53(8):829-40.
5. Kaur A, Kumar S. Plants and plant products with potential antipsoriatic activity-A Review. Pharmaceutical Biology, 50, 2012, 1573-1591.
6. Krueger G, Koo J, Lebwohl M, Menter A, Stern RS, Rolstad T. The impact of psoriasis on quality of life: Results of a 1998 National Psoriasis Foundation patient-membership survey. Archives of Dermatology, 137, 2001, 280-284.
7. Singh NP, Panda H. Meditional herbs with their formulation. DayaPublishiag House, New Delhi, 2005.
8. Arora N, Shah K, Pandey-Rai S. Inhibition of imiquimodinduced psoriasis-like dermatitis in mice by herbal extracts from some Indian medicinal plants. Protoplasma. 2016; 253(2):503-15. doi: 10.1007/s00709-015-0829.
9. Vogel GH. Drug Discovery and Evaluation: Pharmacological Assays, Vol. 2, third ed. Springer, New York, 2008.
10. Na Takuathung M, Wongnoppavich A, Panthong A, et al. Antipsoriatic effects of wannachawee recipe on imiquimodinduced psoriasis-like dermatitis in BALB/c Mice. Evid Based Complement Alternat Med. 2018; 2018:7931031. doi: 10.1155/2018/7931031.
11. Azfar RS, Gelfand JM. Psoriasis and metabolic disease: Epidemiology and pathophysiology. Curr OpinRheumatol. 2008; 20:416–22.
12. Krueger G, Ellis CN. Psoriasis--recent advances in understanding its pathogenesis and treatment. J Am Acad Dermatol 2005; 53:S94-100.
13. Ghoreschi K, Thomas P, Breit S, Dugas M, Mailhammer R, van Eden W et al. Interleukin-4 therapy of psoriasis induces Th2 responses and improves huma
14. Khandelwal KR. Preliminary photochemical screening, in: Practical Pharmacognosy Techniques and Experiments. 8 thedn. Nirali Publication, Pune. 2001, 149-156.
15. SaradhajyothiKoona, Subbarao Budida Antimicrobial potential of the extracts of the leave of Azadirachta indica, Linn. Nat Sci Biol, 2011; 31:65-69.
16. Pulliah T. New Delhi: Regency Publication; 2002. Medicinal Plants in Andhra Pradesh; pp. 132–3.
17. Boligon AA, De Brum TF, Frolhich JK, Froeder AL, Athayde ML. HPLC/DAD profile and determination of total phenolics, flavonoids, tannins and alkaloids contents of scutiabuxifoliareissek stem bark. Res J Phytochem. 2012; 6:84–91.
18. Abubakar AR, Haque M. Preparation of medicinal plants: basic extraction and fractionation procedures for experimental purposes. J Pharm Bioall Sci. 2020;12:1-10.
19. Boehncke WH, Schon MP. Psoriasis. Lancet, 386 (9997), 2015, 983–94.
20. Bosman B, Matshiesen T, Hess V, Frideriche E, A quantitative method for measuring antipsoriatic activity of drugs by the mouse tail test. Skin Pharmacology, 5, 1992, 41-48.
21. Horváth S, Komlódi R, Perkecz A, Pintér E, Gyulai R, Kemeny A. Methodological refinement of Aldarainduced psoriasiform dermatitis model in mice. Sci Rep. 2019; 9(1):3685. doi: 10.1038/s41598-019-39903.
22. Wu HH, Xie WL, Zhao YK, Liu JH, Luo DQ. Imiquimod increases cutaneous VEGF expression in imiquimodinduced psoriatic mouse model. CurrVascPharmacol. 2016;14(3):275-9. doi: 10.2174/1570161114666160106151 837.
23. Lin Y, Yang S, Chen C, Kao H, Fang J, Simon M. Using imiquimod-induced psoriasis-like skin as a model to measure the skin penetration of anti-psoriatic drugs. PLoS One. 2015; 10(9):e0137890. doi: 10.1371/journal. pone.0137890.
24. Organization for Economic Cooperation and Development (OECD) guidelines for acute toxicity of chemicals. No. 425. [Adopted: 3 October 2008]; Schon MP, Boehncke WH. Psoriasis. N Engl J Med. 2005; 352:1899–912.
25. Gibbs S. Skin disease and socioeconomic conditions in rural Africa: Tanzania. International Journal of Dermatology, 35(9), 1996, 633–639.
26. Danilenko DM. Review paper: Preclinical models of psoriasis. Vet Pathol. 2008; 45:563–75.
27. Meeuwis, de Hullu JA, Massuger LF, van de Kerkhof PC, van Rossum Genital psoriasis: A

systematic literature review on this hidden skin disease. Acta DermVenereol. 2011; 91:5–11.
28. Krueger G, Koo J, Lebwohl M, Menter A, Stern RS, Rolstad T. The impact of psoriasis on quality of life: Results of a 1998 National Psoriasis Foundation patient-membership survey. Arch Dermatol. 2001; 137:280–4.
29. Kaur A, Kumar S. Plants and plant products with potential antipsoriatic activity-a review. Pharm Biol. 2012; 50:1573–91.
30. Vjayalakshmi A, Ravichandiran V, Malarkodi V, Nirmala S, Jayakumari S. Screening of flavonoid "quercetin" from the rhizome of *Smilax china Linn*. for anti-psoriatic activity. Asian Pac J Trop Biomed. 2012;2:269–75.
31. Skuric J, Orolic N, Kolaric D, Dikic D, Benkovic V, Knezevic AH, et al. Effectivity of flavonoids on animal model psoriasis-thermographic evaluation. Period Biol. 2011; 57:457–63.
32. Tambekar DH, Dahikar SB. Antibacterial activity of some Indian Ayurvedic preparations against enteric bacterial pathogens, Journal of Advanced Pharmaceutical Technology & Research 2011; 2(1):24-29.
33. Bansod S, Rai M. Antifungal activity of essential oils from Indian medicinal plants against human pathogenic Aspergillus fumigatus and A. niger. World J Med. Sci. 2008; 3(2):81-88.
34. Organization for Economic Cooperation and Development. OECD Guidelines for Acute Toxicity of Chemicals. Paris, France: Organization for Economic Cooperation and Development; 2001.
35. Krueger G, Koo J, Lebwohl M, Menter A, Stern RS, Rolstad T. The impact of psoriasis on quality of life: Results of a 1998 national psoriasis foundation patient-membership survey. Arch Dermatol 2001; 137:280-4.
36. Srivastava AK, Nagar HK, Chandel HS, Ranawat MS. Antipsoriatic activity of ethanolic extract of Woodfordiafruticosa (L.) Kurz flowers in a novel in vivo screening model. Indian J Pharmacol 2016; 48:531-6.
37. Hussein ZA, Al-Zubaidy AA, Sahib HB. The antiangiogenic activity of Phoenix dactylifera seeds methanol extract in vivo study. Iranian J Pharm Sci. 2018;14(2):83-92.
38. Syed TA, Ahmad SA, Holt AH, Ahmad SA, Ahmad SH, Afzal M. Management of psoriasis with *Aloe vera* extract in a hydrophilic cream: A placebo-controlled, double-blind study. Trop Med Int Health 1996; 1:505-9.
39. Kaur CD, Saraf S. Topical vesicular formulations of *Curcuma longa* extract on recuperating the ultraviolet radiation-damaged skin. J Cosmet Dermatol 2011; 10:260-5.
40. Lee SY, Nam S, Kim S, Koo JS, Hong IK, Kim H, et al. Therapeutic efficacies of Artemisia capillaris extract cream formulation in imiquimod-induced psoriasis models. Evid Based Complement Alternat Med. 2018; 2018:3610494.