

Synergistic effect of indigenous medicinal plant extracts on psoriasis

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Abstract

The present paper deals with evaluation of synergistic effect of two medicinal plants, *Pongamia pinnata* Linn and *Psoralea corylifolia* Linn seed extracts on psoriasis. The lack of adequate experimental model is a major impediment in psoriasis research. The development of such a model would, without doubt, contribute to a pivotal breakthrough in understanding the disease and facilitate studies of its underlying causes as well as testing of new therapeutic agents. Extraction was carried out with soxhlet apparatus using the solvent ethanol. Both the extracts were formulated into 5% ointment by using simple ointment as base for topical application on psoriasis induced animals. The anti-psoriatic activity was done by two In vivo methods, those are, Induction of psoriasis in Guinea pig skin by propranolol method where the thickness of epidermis increased by propranolol and Freund's complete adjuvant and Rat UV B ray Photo dermatitis model for psoriasis where the epidermal layer thickness get increased by UV B rays. The significant decrease of epidermal thickness by extract formulations was measured and also histopathological studies were done. *In vitro* anti-bacterial activity was done by disc diffusion method by measuring zones of inhibition and by determination of MIC. Ethanolic seed extracts exhibited significant effect of anti-psoriatic and anti-bacterial activity. Both the seed extracts showed synergistic effect on psoriasis which is concluded by measuring mean thickness of epidermis and histopathological reports and anti-bacterial studies by zones of inhibition and MIC when compared with the individual extracts.

Key Words: *Pongamia pinnata* Linn, *Psoralea corylifolia* Linn, Phytochemical screening, Anti-psoriatic, Anti-bacterial

1.Introduction

Psoriasis is a common, conventional, inflammatory and hyperplastic condition of the skin, involving both environmental and genetic factors. The main symptom of psoriasis is excessive growth and differentiation of epidermal layer which can be cured with appropriate therapy with the possibilities of recurrence. *Pongamia pinnata* plant is used for anti-inflammatory¹ anti-plasmodial, anti-nonciceptive, anti-hyperglycaemics, anti-convulsant², anti-lipoxidative, antidiarrhoeal, anti-ulcer, anti-hyperammonic, CNS depressant activity³ and antioxidant. *Pongamia* Seed oil is also used as insecticidal, bactericidal and nemacidal. Hence the flavonoid is the main constituent of this plant that is useful for the treatment of skin diseases⁴. Powered seeds are used for treatment of leucoderma. A compound ointment of the powered seeds of *Psoralea corylifolia* and *Cassia tora* with lime juice was tried in cases of ringworm with marked beneficial results. *Psoralea corylifolia* fruit is prescribed in stomachache, spermatorrhoea and certain skin diseases. The main constituents in *Psoralea corylifolia* are phenols, coumarins and flavonoids. The coumarin psoralen is the main

compound present in this *Psoralea corylifolia*, responsible for treatment of skin diseases like leucoderma etc⁵. It shows potential anti-bacterial activity⁶. The present study is evaluation of synergistic effect of *Pongamia pinnata* and *Psoralea corylifolia* on psoriasis.

2. Materials and Methods

2.1 Plant Materials: *Pongamia pinnata* Linn and *Psoralea corylifolia* Linn plants (belonging to the family fabaceae) are widely found throughout India. The plant materials were collected from Erode and Tiruchi cities of Tamilnadu respectively and authenticated by botanical survey of India, Coimbatore, Tamilnadu, India. The specimen numbers are P cog plant ID- 2846/08-04-2012 and P cog plant ID- 3201/08-04-2012 respectively.

2.2 Animals: A group of twenty *Albino Guinea Pigs* weighing about 300 – 350 grams each and another group of thirty *Wistar Albino Rats* weighing 150 – 200 grams were used. All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee of NCP and were in accordance with guidelines of the IAEC. Approval was obtained from IAEC (proposal number: NCP/IAEC/No.02/2012-2013).

2.3 Ethanolic Extraction of Seeds of *Psoralea corylifolia* and *Pongamia pinnata*: About 1 kg of *Psoralea corylifolia* and *Pongamia pinnata* seeds were procured in Trichy and Erode respectively, Tamil Nadu and authenticated by the Survey of Medicinal Plants Unit (A Unit of Govt. of India). The cleaned, air dried and crushed seeds were defatted with petroleum ether (60° - 80°C) at room temperature. The defatted seeds were further extracted with 70% ethanol by soxhlation for 12 hrs. The solvent from the extract was completely removed by distillation under reduced pressure. The resulting brown coloured sticky semi solid extract was stored in vacuum desiccators for further use. The successive percentage yields of the ethanolic extracts were 6.2% w/w and 8.5% w/w respectively.

2.4 Phytochemical Screening: The chemical tests were performed for testing of presence of chemical constituents and results were shown in the table no.1

Table 1: Phytochemical constituents present in both seeds (*Pongamia pinnata* and *Psoralea corylifolia* ethanol extract)

Sr.no	Chemical constituent	<i>Pongamia pinnata</i> (ethanol extract)	<i>Psoralea corylifolia</i> (ethanol extract)
1	Carbohydrates	+	+
2	Proteins	-	-
3	Alkaloids	+	+
4	Glycosides	+	-
5	Tannins	-	-
6	Saponins	-	-
7	Flavonoids	+	+
8	Terpenoids	+	-
9	Fixed oils and fat	+	+
10	Phenols	-	+

+ = present, - = absent

2.5 Preparation of ointments: Drug I- 5 % ointment of *Pongamia pinnata* was prepared by mixing 5 mg of extract with 95 mg of simple ointment.

Drug II- 5% ointment of *Psoralea corylifolia* was prepared by mixing 5 mg of extract with 95 mg of simple ointment.

Drug III- 2.5 mg of *Pongamia pinnata* extract and 2.5 mg of *Psoralea corylifolia* were mixed together with 95 mg of simple ointment.

2.6 In vitro Antibacterial Activity

2.6.1 Disc diffusion method : The following strains have been used for study:

Staphylococcus aureus - Gram positive bacteria (ATCC 6538)

Escherichia coli - Gram negative bacteria (ATCC 11775)

A well was prepared in the plate with help of a cork-borer (6 mm), four holes per plate were made in set of agar containing a bacterial culture. A total of 0.2 ml test solution was poured into the wells with the concentration 1000 µg/ml of *Pongamia pinnata*, 1000 µg/ml of *Psoralea corylifolia* and 1000µg/ml of combined extracts (500µg of *Pongamia* and 500µg of *Psoralea*) were prepared in ethanol as well as standard (1000 µg/ml), using a dropping pipette under aseptic condition and labeled accordingly. The plates were maintained at room temperature for 3-5 hrs to allow diffusion of the solution into the medium. The Petri dishes used for anti-bacterial screening were incubated $37 \pm 1^\circ\text{C}$ for 24 hrs. The diameter of zones of inhibition (mm) surrounding each of the wells was recorded. The results were compared to streptomycin (1000 µg/ml) for anti-bacterial activity^{6, 7} and reported in table no.2

2.6.2 Determination of MIC: The MIC values were determined for microorganisms that were found to be sensitive to *Pongamia pinnata* and *Psoralea corylifolia* during the disc diffusion method. To accomplish this, the microorganism inocula were prepared from 12-h broth cultures and the suspensions were then adjusted to a turbidity of 0.5 McFarland. Susceptibility tests were then conducted using the standard broth micro dilution method in MHB with an inoculum of $\sim 5 \times 10^4 \text{CFU mL}^{-1}$. The MHB was then supplemented with serial dilutions of ethonolic seed extracts of *Pongamia pinnata* (PP) and *Psoralea corylifolia* (PC) and combination of two extracts (PP+PC) ranging from 5 to 2000 µg mL⁻¹ and streptomycin concentration was ranging from 0.05 to 250 µg mL⁻¹ [8]. The lowest concentration of PP+PC capable of inhibiting visible growth after 24h of incubation at 37°C was then recorded as the MIC and results are shown in table no.3

2.7 In vivo Anti-Psoriatic activity

2.7.1 Induction of Psoriasis in Guinea Pig Skin by Using Propranolol Method: Twenty albino adult guinea pigs should be divided into 5 groups with 4 animals each. Each animal should be treated with propranolol of 0.1mg per 2 ml saline through oral gavage for thirty days continuously. In addition intradermal injections of propranolol 0.1mg dissolved in 0.25ml saline and emulsified with equal volume of Freund's complete adjuvant. These injections are given intradermally at weekly intervals between the shoulder blades of guinea pigs. This should be done for thirty days. After thirty days there is the development of white patches on the skin with redness^{9, 10, 11}.

And then the animals are divided into five groups.

Group 1: serves as a control which receives simple ointment.

Group 2: serves as standard which is treated with coal tar.

Group 3: serves as test receives Drug I (5% *Pongamia pinnata* ointment)

Group 4: serves as test receives Drug II (5% *Psoralea corylifolia* ointment)

Group 5: serves as test receives Drug III (5% of combination ointment)

Application of the drugs was started after one month of the induction and was continued for 30 days. On the 30th day, 2 hours after the last treatment, animals were anaesthised using anaesthetic ether and the exposed area of the skin was removed by surgical incision. The incised skin was then fixed in 10% buffered formalin solution that was followed by gradual dehydration using increasing strength of alcohol (80% to absolute alcohol). The skin was then embedded in paraffin wax and sections of 4 µm thickness were obtained using a microtome. The skin thickness measurements were made at a magnification of 400X using Olympus microscope having a digital camera attachment and software to take measurement and given for histopathological studies. The results were shown in the following table 4 and figure 1.

2.7.2 Rat Ultraviolet Ray B Photo Dermatitis Model on Psoriasis: Wistar rats weighing around 300 g are used. Hair on the dorsal skin is clipped and carefully shaved. An area (1.5 × 2.5 cm) on one side of the flank is irradiated for 15 min at a vertical distance of 20 cm with UV-B lamps. A biphasic erythema is 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 and 48 h. The color is brownish-red, and the reaction is confined to the exposed area with a sharp boundary. By 48–72 h after irradiation, dark-brown scale is formed on the erythematous lesion. Pieces of the scale are relatively thick.

Then the animals are divided into 5 groups with 6 animals each.

Group 1: serves as a control which receives simple ointment.

Group 2: serves as standard which is treated with coal tar.

Group 3: serves as test receives Drug I (5% *Pongamia pinnata* ointment)

Group 4: serves as test receives Drug II (5% *Psoralea corylifolia* ointment)

Group 5: serves as test receives Drug III (5% of combination ointment)

Application of the drug was started 12 hours after irradiation, and was continued for three days. A Schedule of two applications per day spaced over 12 hours interval was maintained. On the third day, 2 hours after the last treatment, animals were anaesthised using anaesthetic ether and the exposed area of the skin was removed by surgical incision. The incised skin was then fixed in 10% buffered formalin solution that was followed by gradual dehydration using increasing strength of alcohol (80% to absolute alcohol). The skin was then embedded in paraffin wax and sections of 4 μm thickness were obtained using a microtome [12]. The skin thickness measurements were made at a magnification of 400X using Olympus microscope having a digital camera attachment and software to take measurement and given for histopathological studies. The results were shown in the following table 5 and figure 2.

3. Results and Discussion

3.1 *In vitro* Anti-bacterial activity

3.1.1 Disc diffusion method: Anti-bacterial activity was done by disc diffusion method and the activity of the extracts was measured by zone of inhibition of microbes on the plate with agar medium and reports were given in table 2.

Table 2: Measurement of Zone of inhibition

Micro organism	Zone of inhibition (mm)			
	Pongamia pinnata (1000 $\mu\text{g/ml}$) (PP)	psoralea corylifolia (1000 $\mu\text{g/ml}$) (PC)	Combination both (1000 $\mu\text{g/ml}$) (PP+PC)	Streptomycin (1000 $\mu\text{g/ml}$)
Staphylococcus aureus	15	18	23	25
Escherichia coli	14	19	21	24

Hence, both the plant extracts showed the synergistic action by showing more zone of inhibition when compared to the individual plant extracts.

3.1.2 Determination of MIC: The MICs of the PP, PC, PP+PC against the strains of *Staphylococcus aureus* and *Escherichia coli* are shown in Table 3. The MICs determined using the broth dilution method confirmed the results obtained using the disc diffusion method. PP+PC showed synergistic anti-bacterial activity against each of the tested strains, and these values ranged from 100 to 650 $\mu\text{g mL}^{-1}$.

Table 3: Measurement of MIC of extracts and streptomycin

Micro Organisms	MIC ($\mu\text{g/ml}$)			
	PP extract	PC extract	PP+PC	Streptomycin
Staphylococcus aureus (ATCC 6538)	1000	900	500	250
	850	650	250	50
	800	700	100	10
	950	500	150	25
Escherichia coli (ATCC 11775)	1050	950	650	500
	900	850	500	550
	850	700	250	200
	1000	800	600	150

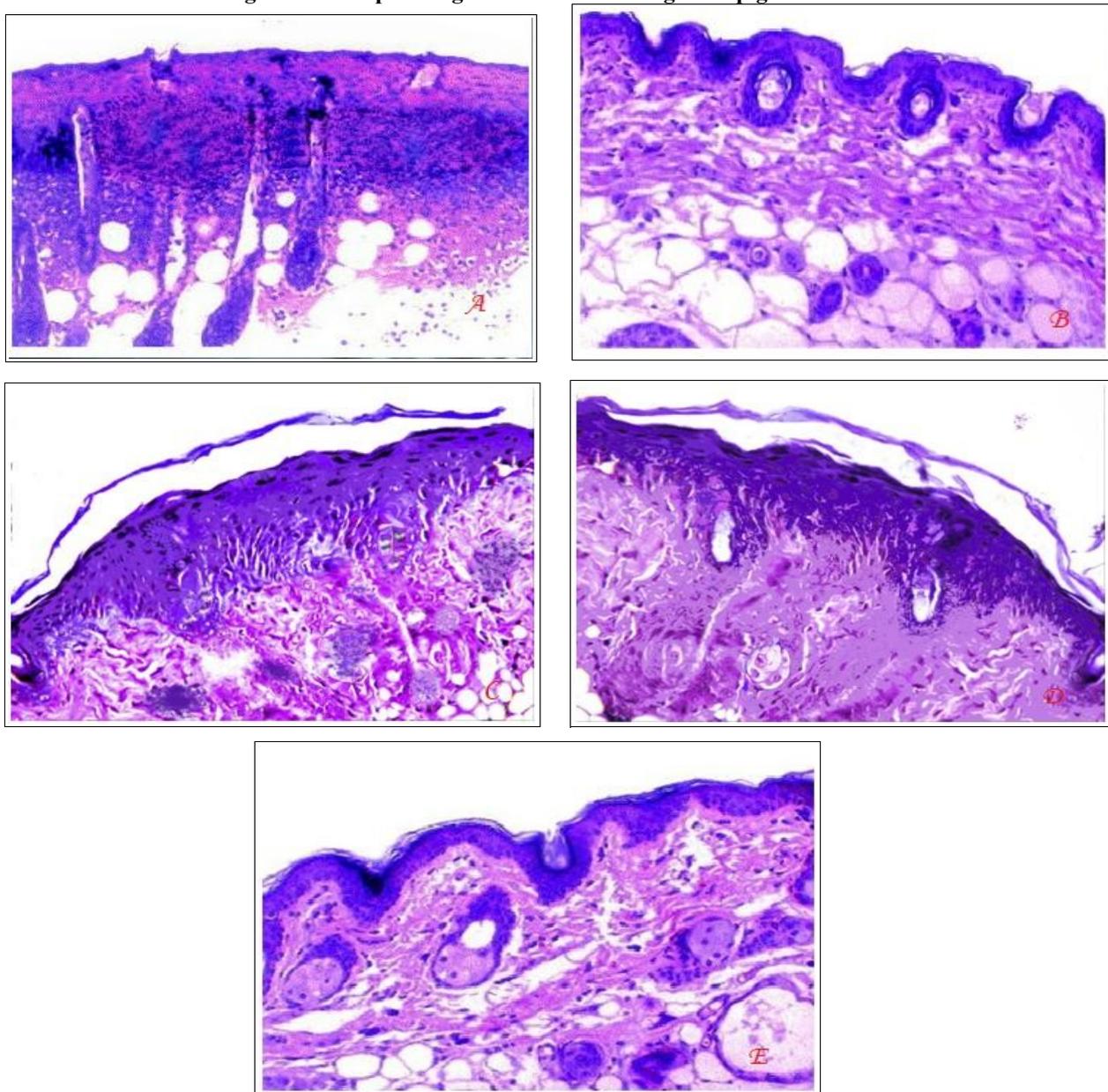
3.2 *In vivo* Anti-Psoriatic activity

3.2.1 Induction of Psoriasis in guinea pig skin by propranolol method: The ability of β -adrenergic blocking agents to induce psoriasis as an adverse effect prompted us to use such an agent to induce psoriasis in guinea pigs. Among the drugs that may induce psoriasis, an important place is held by the β -adrenergic blocking drugs. The ability of β -adrenergic blocking agents to induce psoriasis prompted several investigators to exploit this adverse reaction to develop an animal model for psoriasis. Hence the propranolol was used to induce psoriasis in guinea pigs along with Freund's adjuvant [9, 10]. Freund's adjuvant complete is a solution of antigen in essential oil and used as a potentiator of immune system. Freund's complete adjuvant is effective in stimulating immunity and may lead to the potentiation of the production of certain immunoglobulin. The intradermal administration of Freund's adjuvant along with propranolol induces psoriasis locally. Application of the drugs was started after one month of the induction and was continued for 30 days. On the 30th day, 2 h after the last treatment, animals were anaesthised using anaesthetic ether and the exposed

area of the skin was removed by surgical incision. The skin thickness measurements were made at a magnification of 400X using Olympus microscope having a digital camera attachment and software to take measurement and given for histopathological studies. The results were shown in the following table 4 and figure 1.

Mean thickness of the epidermis, Group 1 (treated with simple ointment), Group 2 (treated with coal tar), Group 3 (Drug I- 5% *Pongamia pinnata*), Group 4 (Drug II- 5% *Psoralea corylifolia*), Group 5 (Drug III- 5% of combination) were found to be 94.62 ± 3.04 , 46.7 ± 3.81 , 73.42 ± 1.92 , 68.2 ± 2.23 , 55.17 ± 2.42 respectively. There was significant decrease of epidermal thickness of Group 5 when compared to Group 1^{***} ($p < 0.001$) and Group 3^{**} ($p < 0.01$) and Group 4^{*} ($p < 0.05$). There was no significant change between Group 5 and Group 2. It determines that the effect of combination extracts was probably equal to standard drug coal tar. The histopathological reports also proved that the ethanolic extracts of *Pongamia pinnata* and *Psoralea corylifolia* showed the synergistic effect by reducing the keratinocytes when compared to other extracts.

Figure 1: Histopathological observations of guinea pig skin sections

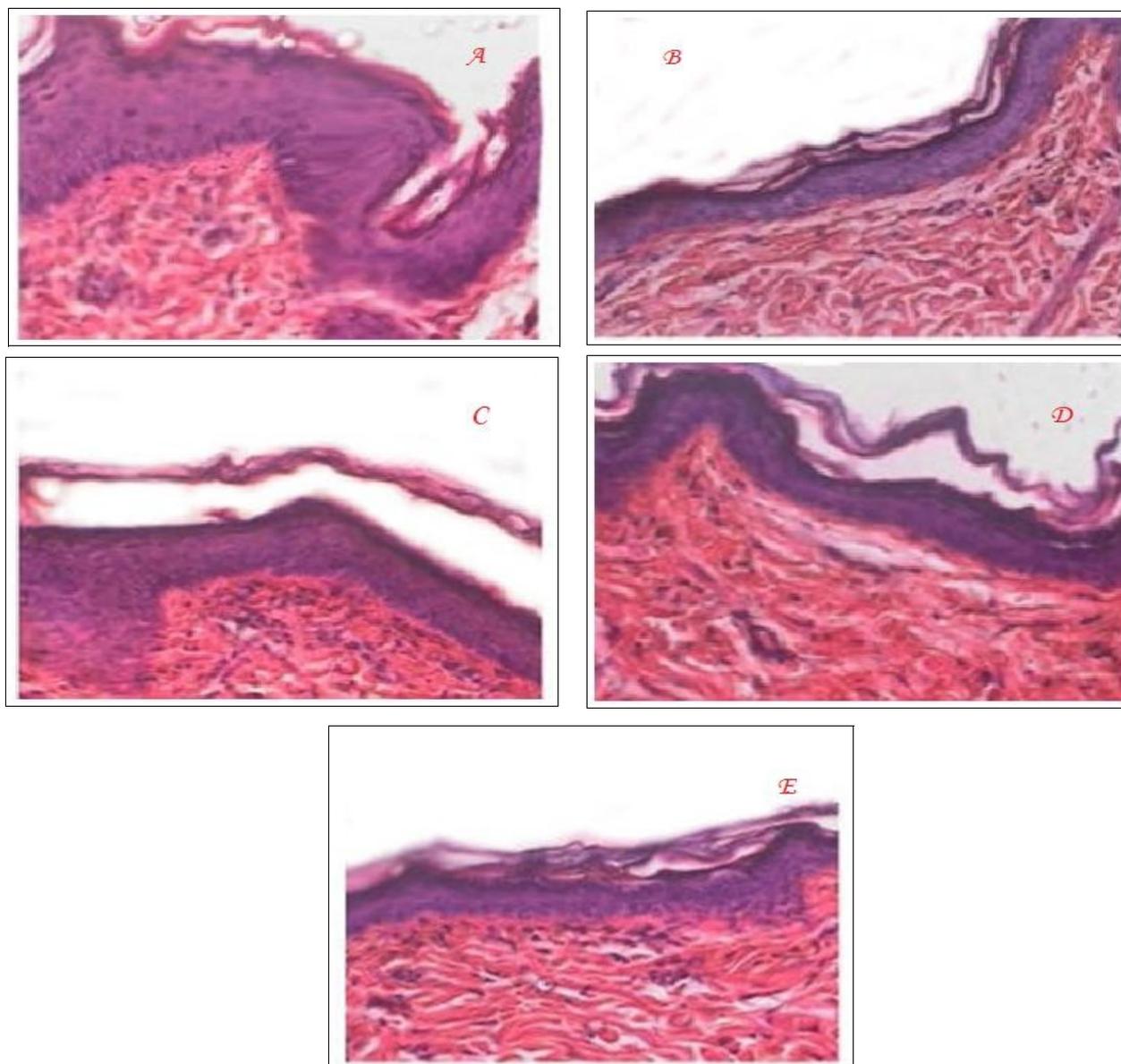


A- Simple ointment, B- Coal tar, C- Drug I, D- Drug II, E- Drug III

3.2.2 Rat Ultraviolet Ray B Photo Dermatitis Model on Psoriasis: Psoriasis was induced in albino rats by passing UV B radiation on the particular area of the skin. By exposing rat skin to UV B rays produce increased layers of

keratinocytes (psoriasis) and then treated with plant extract ointments and the skin was removed by surgical incision. These sections were transferred on to a glass slide and stained with hematoxylin and eosin. The skin thickness measurements were made at a magnification of 400X using Olympus microscope having a digital camera attachment and software to take measurement and given for histopathological studies. The results are shown in the following table 4 and figure 2.

Figure 2: Histopathological observations of albino rat skin sections



A- Simple ointment, B- Coal tar, C- Drug I, D- Drug II, E- Drug III

Mean thickness of the epidermis, Group 1 (treated with simple ointment), Group 2 (treated with coal tar), Group 3 (Drug I- 5% *Pongamia pinnata*), Group 4 (Drug II- 5% *Psoralea corylifolia*), Group 5 (Drug III- 5% of combination) were found to be 71.1 ± 2.05 , 32.38 ± 1.71 , 55.8 ± 1.78 , 49.9 ± 0.81 , 39.8 ± 1.3 respectively. There was significant decrease of epidermal thickness of Group 5 when compared to Group 1^{***} ($p < 0.001$) and Group 3^{***} ($p < 0.001$) and Group 4^{*} ($p < 0.05$). There was no significant change between Group 5 and Group 2. It determines that the effect of combination extracts was probably equal to standard drug coal tar. The histopathological reports also proved that the ethanolic extracts of *Pongamia pinnata* and *Psoralea corylifolia* showed the synergistic effect by reducing the thickness of epidermis when compared to other extracts.

Table 4: Measurement of Total epidermal thickness in both in vivo methods of anti-psoriatic activity

Experiment	TOTAL EPIDERMAL THICKNESS(μm) (Mean \pm S.E.M)				
	Group 1	Group 2	Group 3	Group 4	Group 5
1. Induction of psoriasis in guinea pig skin by propranolol method	94.62 \pm 3.04	46.7 \pm 3.81	73.42 \pm 1.92	68.2 \pm 2.23	55.17 \pm 2.42***
2. Rat UV B ray Photodermatitis model	71 \pm 2.05	32.38 \pm 1.7	55.8 \pm 1.78	49.9 \pm 0.81	38.9 \pm 1.2***

*** Significant ($P < 0.001$), Group 5 when compared to Group 1.

4. Conclusion

The plants *Pongamia pinnata* Linn and *Psoralea corylifolia* Linn belonging to family fabaceae were selected for evaluating the synergistic effect of both plants on anti-bacterial and anti-psoriatic activities. The pharmacological evaluation of psoriasis and antibacterial studies were performed. From the above results it was proved that the seed extracts of *Pongamia pinnata* and *Psoralea corylifolia* had synergistic action on anti-bacterial and anti-psoriatic studies.

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