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Anti – Psoriatic Activity of *Psorospermum Febrifugum* Stem Bark Extract Using the Rat – Dinitrofluorobenzene Induced Model

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Abstract— *Psoriasis has been described as a chronic inflammatory skin disease. Psorospermum febrifugum is a flowering plant and belong to the family Hypericaceae and found in the tropical areas of the world such as Africa, South America and Madagascar. Alcoholic and aqueous Extracts of P. febrifugum have been reportedly used to treat various skin diseases. This research was therefore conducted to investigate the effectiveness of P. febrifugum stem bark extract against psoriasis. The rat model of psoriasis was developed by induction for 5 days using 100 µL of 0.5% hapten – 2, 4 – dinitrofluorobenzene in acetone and olive oil in 4:1 ratio. Animals were divided into 5 groups and were administered various doses of the extracts and retinoic acid except the control groups. Animals were also challenged by topical application of 20µLof 0.2% dinitrofluorobenzene in a mixture of acetone and olive oil to both sides of the ear on days 8, 9 and 10. 400 mg/kg body weight dose significantly reduced psoriatic lesion by 93.15% as against 61.21% by retinoic acid. Ear weight was decreased 54.71% by 200 mg/kg, 71.69% by 400 mg/kg and 69.78% by retinoic acid. The percent inhibitions of ear thickness were 61.76 and 89.71 respectively for 200 and 400 mg/kg body weight of the extract and 63.23 for 0.5 mg/kg of retinoic acid. These results compared to the reported effectiveness of other plant extracts 62 – 65.5% and P. febrifugum with 71 – 93.15%, showed serious indication that P. febrifugum could be effectively used to cure psoriatic conditions.*

Keywords— *Psoriasis; Anti – psoriatic; Dinitrofluorobenzene; Psorospermum febrifugum; Retinoic acid*.

I. INTRODUCTION

Psoriasis has been described as a chronic life-long inflammatory disease of the skin. It has also been known to affect the knees, elbows, scalp, eye and clinically characterized by erythematous, red, and rounded plagues covered by silvery scales [1,2,17,20]. Skin ailments like dermatitis, angioedema and Psoriasis among others are auto-immune disorder and are characterized by chronic inflammation and proliferation of cells[3]. The severity of psoriatic conditions raised much interest among scientists, making it an important research subject. Till date, no drug has been found to effectively cure psoriasis and other dermatological diseases. Moreover, many of them are expensive and produce toxic side effects[4] while natural remedies are less toxic and seem promising[5]. The exact causes of psoriasis are not known but the immune system and heredity is revealed to be a major factor in its development. Report shows that the inflammatory processes are immune-responses and are initiated and maintained by T helper cells in the dermis[6].

The importance of recognizing that medications may be the cause of either the eruption or induction (Table 1)[2] of psoriasis must be acknowledged. Many authors documented the relationship between certain medications and the presence of psoriasis. Report showed that within a small population of 63 patients, medications could be responsible for psoriasis in as many as 83% of cases[7]. Some medications reported to initiate or increase already existing psoriasis include beta blockers, lithium, chloroquine and gold salts[8]. Other researchers as well found that chloroquine, beta adrenergic antagonists, corticosteroids and indomethacin either induce or exacerbate psoriasis[9].

Hapten – 2, 4 – dinitrofluorobenzene is a strong sensitizing agent. Topical application of the chemical induces a T-cell-mediated inflammation of the skin[10] and erythematous lesion and other symptoms similar to those observed in psoriasis.

Natural polyphenols found in most plants are good antioxidants and as well multifunctional moieties which have anti-inflammatory and antiproliferative properties. They act by modulation of multiple signaling pathways. Polyphenols are known to have a wide range of biological activities such as immunomodulatory properties, antitumor, antimicrobial, anti-inflammatory and oxygen radical scavenging properties[11].

The present study evaluates the anti-psoriatic activity of *psorospermum febrifugum* stem bark extract. The plant *P. febrifugum* spach commonly called "Christamss berry" is a flowering plant of the family *Hypericaceae*. It is a shruh or small tree found in the tropical regions of Africa, Madagascar and South America. Majority of the plants under the genus *psorospermum* have a long history for their use in folk-loric medicine of indigenous African populations as a purgative, antidote against poisons, febrifuge, and as a remedy for leprosy, subantanceous wounds and other skin diseases[12].

TABLE 1. Drugs responsible for the induction of psoriasis.

Acetazolamide	Atenolol	Hydroxychloroquine
Aminoglutethimide	Chloroquine	Indomethacin
Amiodarone	Cimetidine	Lithium
Amoxicillin	Corticosteroids	Methicillin
Ampicillin	Cyclosporine	Penicithris
Aspirin	Diclofenac	Potassium Iodide
	Diltiazem	Propranolol Terbinafine

II. MATERIALS AND METHODS

2.1 Animals

Swiss albino rats 200 – 250g were used for the experiments. The animals were sourced from the Department

of Zoology and Environmental Biology, University of Nigeria, Nsukka, Enugu State, Nigeria. They were kept in cages of five animals per group to acclimatize and room temperature maintained at 20-25^{o}C on 12h light/dark cycle. Food and water were given before the experiment. Experiments were performed adhering to the International Guide for the Care and Use of Laboratory Animals.

2.2 Drugs and Chemicals

2, 4-dinitrofluorobenzene (DNFB) sigma Aldrich $\geq$ 99% purity was ordered from USA and was supplied by Bristol Scientific Limited, Lagos, Nigeria. Retinoic acid made by Nutricarp International, Canada and Goya Olive Oil by Andalucia, Spain were purchased from Vegil Pharmacy, Nsukka, Enugu State, Nigeria. Acetone and ethanol (sigma Aldrich) were bought from Germany. All the chemical and other reagents were of analytical grade.

2.3 Plant Materials

The plant was collected from a bush at Nsukka, South East, Nigeria in November, 2015 and identified by a taxonomist at the International Centre for Ethnomedicine and Drug Development Nsukka, Enugu State, Nigeria. A voucher specimen was deposited at the centre's Herbarium (Voucher No: INTERCEDD/16024).

2.4 Preparation of Plant Extract

The bark was separated from the wooden stem and air dried for about 14 days and pulverized into coarse powder using Thomas Willy Laboratory Mill Model 4. About five hundred grams of the powder were macerated in 95% ethanol and extracted for 7 days with occasional agitation. The mixture was filtered first with chess cloth followed by filter paper and evaporated under reduced pressure using rotary evaporator (Buchi; CH – 9230 Switzerland)

2.5 Acute Toxicity Studies:

The acute toxicity studies of the extract was carried out according to the method[13] described by Mahesh S. S. *et al* (2013) with slight modifications. Mice approximately 25-30g were divided into groups (n = 5). Animals were treated with several doses of the extract; 10 mg/kg body weight of extract up to the dose of 5000 mg/kg body weight. They were monitored for clinical signs and mortality. All animal experiments were conducted in compliance with NIH Guidline for Care and Use of Laboratory Animals (Pub. number: 05-23 revised 1985) as approved by the University of Nigeria, Nsukka.

2.6 Design for DNFB – Induced Psoriasis Model

The protocol followed a previously described method[14] with some modifications. The dorsal skin of adult albino rats was shaved using depilatory equipment before the experiment. Animals were divided into five groups (n = 5).
Group 1: served as vehicle control (acetone – olive oil was applied to the shared abdomen).
Group II: served as the negative control, induced without treatment.
Group III: treatment group, received extract, 200 mg/kg body weight.
Group IV: treatment group, received extract, 400 my/kg body weight.
Group V: positive control, received 0.5 mg/kg body weight of retinoic acid.

Induction was achieved by repeated application of 100μl of 0.5% dinitrofluorobenzene in acetone – olive oil in the ratio of 4:1 v/v to the shaved abdomen on days 1, 2, 3, 5 and 7. On days 9, 10 and 11 animals were challenged by topical application of 50 μl of 0.2% dinitrofluorobenzene in acetone-olive oil in the ratio of 4:1 to both sides of the ear. Experimental groups except the controls were orally dosed 200 mg/kg and 400 mg/kg of extract for 14 days. 24hrs post treatment, animals were sacrificed and skin samples excised for histological examination. Ear thickness and weight were as well recorded.

2.7 Histological Analysis

Skin samples were excised from the rats and fixed with 10% formaldehyde, embedded in paraffin wax, routinely processed and then sectioned into 5- μm- thick slides. The skin sections were then stained with hematoxylin-esoin and examined by light microscopy for the presence of inflammed cells[15]. The percent inhibition of epidermal thickness, ear weight and thickness was calculated according to the equation:

$$\% \text{ inhibition} = \frac{X_N - X_{DNFB}}{X_N - X_P} X \frac{100}{1}$$

Where X_N = Scores obtained from the disease model (negative control)
X_{DNFB} = Scores obtained from DNFB induced plus *P. febrifugum* treated animals.
X_p = Scores obtained from vehicle control group.

2.8 Statistical Analysis

The results were analyzed using a one way analysis of variance (ANOVA) and data presented as mean $\pm$ standard deviation (SD). The Multiple *post-hoc* differences were assertained by the scheffe test. The $P \leq 0.05$ were considered to be statistically significant.

III. RESULTS

3.1 Acute Oral Toxicity Studies

There was no death or clinical signs recorded during the period of experiment. Hence, the extract is safe and has a wide range of effective dose.

3.2 Anti-Psoriatic Assay

The cutaneous application of 0.5% Dinitrofluorobenzene on the rat's skin produced erytherma. Repeated application showed a significant increase on epidermal thickness and scaling of the rat's skin when compared to the vehicle control where the application of the vehicle (acetone-olive oil 4:1) did not produce any skin differentiation. The thickness of the epidermis was measured to assess the severity of the epidermal hyperplasia caused by DNFB. Epidermal thickness

following histological examination revealed significant increase (two to three times) in the negative control group (Fig. 1 and Table 2) which was induced without treatment.

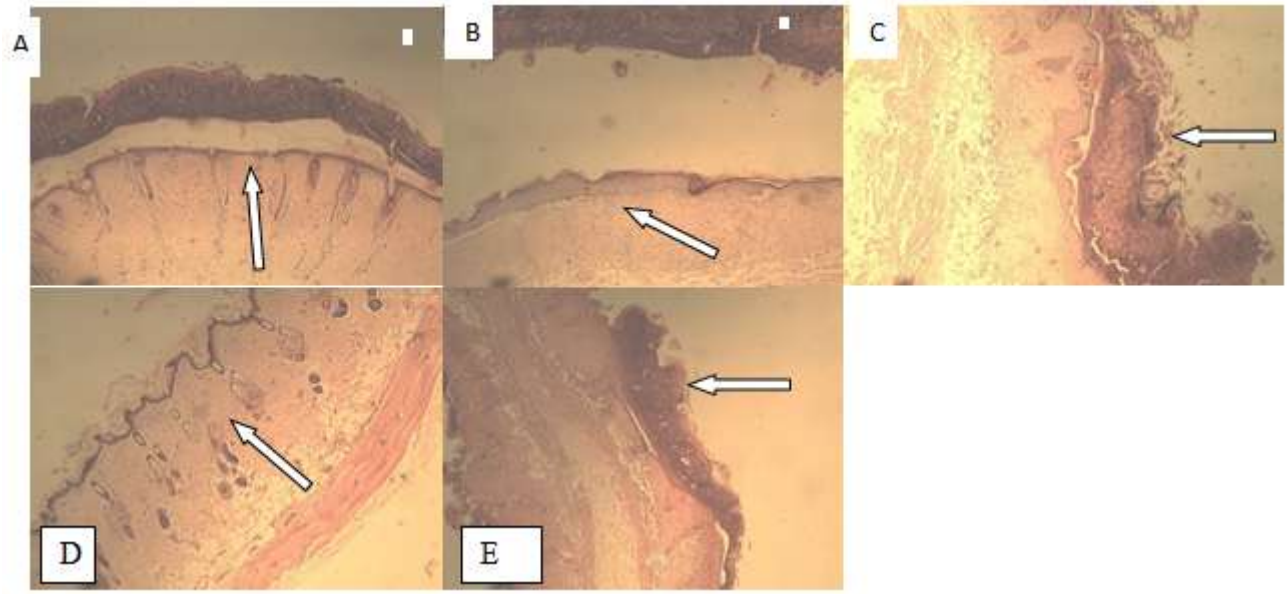


Fig. 1. Photomicrograph of the skin showing the histopathological features of DFNB induced Psoriasis in rats. A = skin of rat treated with 400 mg/kg extract, B = treated with retinoic acid (positive control), C = untreated (negative control), D = uninduced (vehicle control) and E = treated with 200 mg/kg body weight of extract.

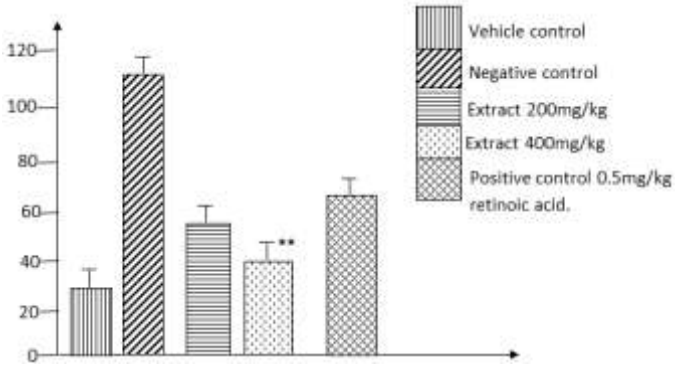
TABLE 2. Anti-psoriatic effect of *P. febrifugum* stem bark ethanol extract on epidermal thickness (μm), ear weight (mg) and ear thickness μm of rat induced by repeated application of DNFB.

Parameter	Vehicle Control	Negative Control	Extract 200mg/kg	Extract 400mg/kg	Positive Control 0.5 mg/kg
Epidermal thickness (μm)	34.00 ± 6.00	111.333 ± 11.72	58.40 ± 2.60**	39.30 ± 1.76*	64.00 ± 13.49
Ear weight (mg)	78.60 ± 0.71	242.00 ± 13.20	152.60 ± 11.20Ñ	124.86 ± 0.33Ñ	128.42 ± 0.66
Ear thickness (μm)	0.68 ± 12.06	2.04 ± 16.09	1.20 ± 0.60**	0.82 ± 1.76Ñ	1.18 ± 6.20

* No significant difference P < 0.05 compared to the vehicle control, **No significant difference P < 0.05 compared to the positive control. Ñ - Significantly lower than the negative control. Data presented as mean ± SD (n = 5)

TABLE 3. Percentage (%) anti-psoriatic effect of 200 and 400 mg/kg of *P. febrifugum* ethanol extract and retinoic acid 0.5 mg/kg body weight on rats induced by repeated application of 0.5% 2, 4-dinitrofluorobenzene.

Parameter	Extract 200 mg/kg	Extract 400 mg/kg	Positive Control Retinoic Acid 0.5 mg/kg
Epidermal thickness (%)	68.45	93.15	61.21
Ear weight (%)	54.71	71.69	69.78
Ear thickness (%)	61.76	89.71	63.23



** No significant difference P < 0.05 compared to the vehicle control

Fig. 2. Effect of *P. febrifugum* stem bark ethanol extract and retinoic acid on epidermal thickness of rat induced by DNFB.

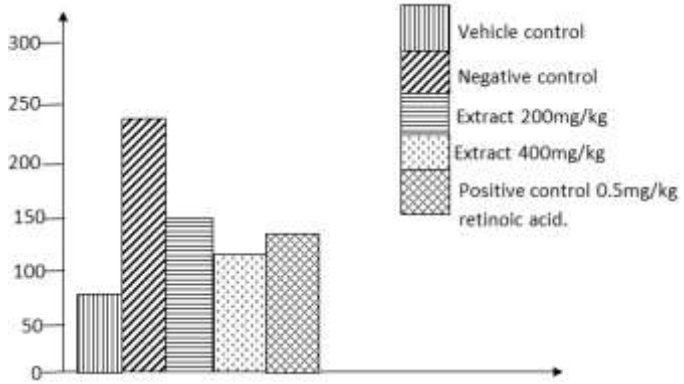


Fig. 3. Inhibition of DNFB- induced ear weight in rats by stem bark ethanol extract of *P. febrifugum* and retinoic acid.

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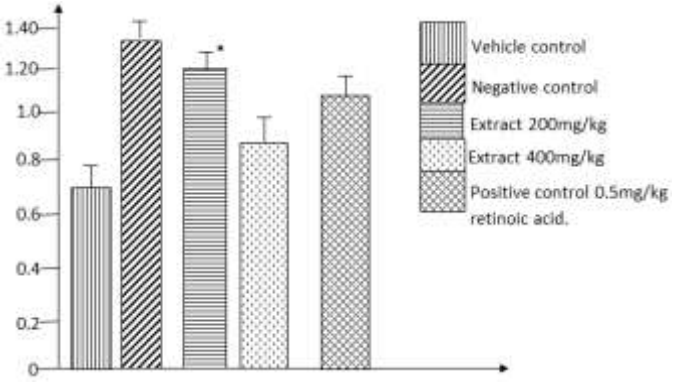

*No significant difference P < 0.05 compared to the positive control

Fig. 4. Reduction of DNFB-Induced ear thickness (μm) in rats by stem bark ethanol extract *P. febrifugum* and retinoic acid

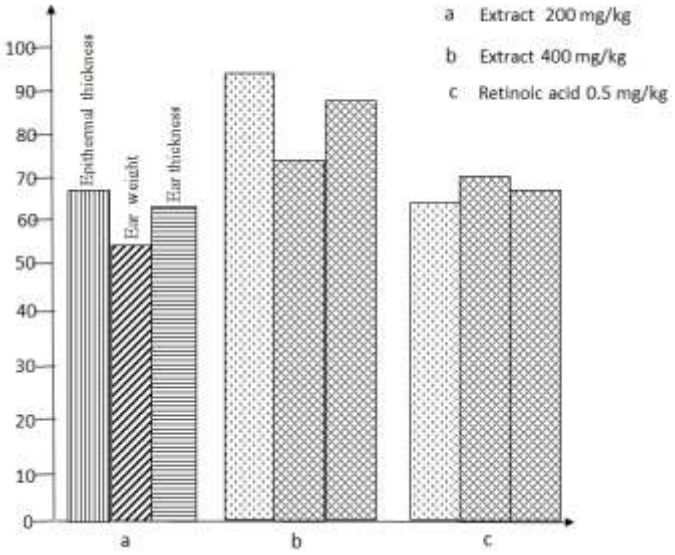

Fig. 5. Percent (%) Inhibition effects of rat – DNFB Induced epidermal thickness (μm) , ear weight (mg) and ear thickness (μm) by stem bark ethanol extract of p. febrifugum and retinoic acid.

The percent inhibition effects on epidermal thickness, ear weight and ear thickness by different doses of the extract were 68.45 for 200, 93.15 for 400 mg/kg body weight and 61.21 for retinoic acid (Table 3 and Fig. 5).

IV. Discussions

Chronic psoriasis was induced in rat by repeated application of 2, 4-dinitrofluorobenene. The psoriasis thus induced was characterized by skin oedema, pronounced differentiation and inflammation.

For the first time, we evaluate and report the anti-psoriatic effects of *psorospermum febrifugum* extract against chronic DNFB-induced psoriasis in rat. Oral administration of ethanol extract of *P. febrifugum* stem bark significantly reduced epidermal thickness, ear thickness and ear weight.

There have been many literature reports for the use of *P. febrifugum* in treatment of various skin diseases.[12] but non have reported on its use in the treatment of psoriasis. The stem bark ethanol extract of *P. febrifugum* showed 93.15% reduction of epidermal thickness at the dose of 400 mg/kg body weight and was revealed to have more activity than retinoic acid (0.5 mg/kg) used as the positive control which showed 61.21% effectiveness. This result agrees with several other reports by those who used retinoic acid as their positive control. Vijayalakshmi A. *et al* [16] (2014) reports the anti-posriatic properties of flavonoids isolated from *Cassia tora* leaf. Their result showed that ethanol extract of *C. tora* leaves had 61.03% activity at 400 mg/kg. The ethanol extract of *Nigella sativa* seed according to Lalitha P. D *et al* [19] (2013) showed 65.01% activity using the mouse tail model.

Comparably, researchers who used DNFB-induced model of psoriasis [9, 12, 13] reports that its repeated application significantly raised epidermal thickness to two or three times the original size. This report agrees with the result of our experiment which increased the thickness of the epidermis from 34.00 (μm) to 111.33 (μm) approximately three times. Rayesh *et al* [18] (2015) using oxazolone-induced model of psoriasis reports 62.2%, 69.2% and 65.5% inhibition of ear thickness, ear weight and epidermal thickness respectively and these values were below the activity of *P. febrifugum* which were 89.71%, 71.69% and 93.15% respectively at 400mg/kg body weight ($P \leq 0.05$). Again, B. Narin Rayesh *et al*[19] (2013) reports that 2% extract of *Tribulus terrestris* inhibited ear thickness, ear weight and epidermal thickness by 73.7, 73.6 and 73.0 percent respectively using oxazolone induced mouse model. These values were still below the results obtained for *P. febrifugum* extract.

V. Conclusion

Psorospermum febrifugum did not produce any toxic effects even at high dose of 5000 mg/kg body weight and showed very high anti-psoriatic activity. This agrees with literature report and the local use of the plant extract in treatment of various skin diseases. However, no earlier report available to us on its use in the treatment of psoriasis. Compared to the effectiveness of other plant extracts in the treatment of psoriatic conditions; 62 – 65.5%, its high effectiveness ranging from 71 – 93.15% strongly indicate that the plant extract could effectively cure psoriasis.

Conflict of Interests

There are no conflicts of interest among the authors.

Acknowledgement

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