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Inhibition of Hapten-2, 4-dinitrofluorobenzene Induced Psoriasis in Rats by Ethanol Extract of *Harungana madagascariensis* (Lam-Poir) Leaf

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ABSTRACT

Psoriasis is a chronic inflammatory skin disorder that is clinically characterized by erythematous, red, sharply demacerated papules and rounded plaques covered by silvery scales. Harungana madagascariensis (Hypericaceae) is a specie of flowering plants found in tropical Africa. Different parts of H. madagascariensis have been used in folk medicine for the treatment of a wide spectrum of human and livestock diseases including skin disorders. This research was therefore conducted to investigate the effectiveness of H. madagascariensis leaf 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 (DNFB) in acetone – olive oil (4:1). Animals were divided into five groups: Group I served as vehicle control (shaved abdomen without induction, only the vehicle was applied), Group II served as the negative control (shaved and induced without treatment), Group III and IV were induced and received 200 and 400 mg/kg body weight ethanol extract respectively and Group V served as positive control and received 0.5 mg/kg body weight retinoic acid. Animals were also challenged by application of 20 µL of 0.2% DNFB in a mixture of acetone and olive oil (4:1) to both sides of the ear on days 8, 9 and 10. The results obtained showed that the extract at 400 mg/kg body weight significantly reduced psoriatic lesion induced by cutaneous application of 2, 4-dinitrofluorobenzene in rats by 74.63%. Comparably, the extract showed higher activity than retinol palmitate (retinoic acid) (0.5 mg/kg), which showed 61.21% reduction. There was a remarkable decrease in ear weight; 66.21% for 200 mg/kg, 76.51% for 400 mg/kg and 56.27% for retinoic acid. The percent inhibitions of ear thickness were 49.46 and 64.52 respectively for 200 mg/kg and 400 mg/kg of the extract and 33.33 for retinoic acid. The results therefore, support the use of H. madagascariensis for the treatment of skin diseases.

Key words: Psoriasis, Inhibition, Dinitrofluorobenzene, *Harungana madagascariensis*, Retinoic acid.

INTRODUCTION

Medicinal plants are known to be safe for human health and are widely employed by the traditional healers for the treatment of various diseases including psoriasis [1]. They are rich in chemical compounds and have attracted researchers' attention towards finding new treatment for psoriasis[2]. Natural polyphenols recognized as potent antioxidants, are multifunctional molecules that can act as anti-inflammatory and anti-proliferative agents through the modulation of multiple signaling pathways. They possess a broad spectrum of biological activities such as immune system activities, oxygen radical scavenging, antimicrobial, anti-inflammatory, and antitumor activities [3].

Psoriasis is a chronic inflammatory skin disorder that is clinically characterized by erythematous, red, sharply demacerated papules and rounded plagues covered by silvery scales [4]. These symptoms depend on the type of psoriasis and the severity. Presently, the exact cause of psoriasis is not known but genetic, immunological and environmental factors [5] are vindicated to play major roles in its development. Psoriatic symptoms manifest when the immune system sends out faulty signals resulting in the speeding-up of the skin cell's (growth) cycle [6]. This faulty signal causes over production of new skin cell known as hyper-proliferation.

Several research suggests that the inflammatory mechanisms are immune based and most likely initiated and maintained primarily by T - cells in the dermis [7]. T cell reactivation in the dermis and epidermis and the local effect of cytokines such as tumor necrosis factor lead to the inflammation and epidermal hyper-proliferation observed in patients with psoriasis.

The disease is a life-long inflammatory disorder that primarily affects the skin, musculoskeletal system, gastrointestinal system and the eye[8]. It is a common problem and millions of people in the world have psoriasis[9]. Pustular psoriasis is characterized by blisters of non-infectious pus that appear on the skin. It is triggered by medications, infectious stress or exposure to certain chemicals. Hapten-2, 4-dinitrofluorobenzne is a

strong sensitizing agent. Topical application of the chemical induces a cytotoxic (Tc) T-cell-mediated antigen-specific type of skin inflammation [10] and erythematous lesion all symptoms very similar to those observed in psoriasis.

H. madagascariensis (*Lam-poir*) is a specie of flowering plant in the family *Hypericaceae* and the sole member of the genus *Harungana*. It is found in tropical Africa. Preparations from different parts of *H. madgascariensis* have been used in African folk-loric medicine for the treatment of a wide spectrum of human and livestock diseases [11]. The red juice from the leaves and stem bark are used as antihaemorrhage during child birth [11], boiled water decoction of the root is used as an antidote in cases of liver and kidney poisoning[11] while the unopened buds are used in the treatment of skin diseases, anaemia, parasitic infestations and wound infections[12]. In the present research, we investigated the inhibition effects of *H. madagascariensis* leaf extract on the psoriatic lesion induced chemically in albino rats.

MATERIALS AND METHODS

DRUGS AND CHEMICALS

2, 4-dinitrofluorobenzene (DNFB) ≥99% purity was ordered from USA and was supplied by Bristol Scientific Limited (Sigma Aldrich Rep) Lagos, Nigeria. Retinol palmitate (retinoic acid) produced by Nutricorp International Canada, and Goya Olive oil by Andalucia, Spain were purchased from Vegil Pharmacy, Nsukka, Enugu state, Nigeria. Other chemicals; acetone, and ethanol were bought from Germany (Sigma Aldrich). All the chemicals and reagents were of analytical grade.

PLANT MATERIALS

The leaves of *Harungana madagascariensis* (*Lam-poir*) were collected in November, 2015 from Ibagwa-Aka, Igbo-Eze South Local Government Area of Enugu State, Nigeria. The identity of the specimen was confirmed and authenticated by Mr. Alfred Ozioko of the International Centre for Ethnomedicine and Drug Development (INTERCEDD) Nsukka, Enugu

State Nigeria. A voucher specimen (INTERCEDD/ 063) was deposited at the centre's Haberium.

PREPARATION OF PLANT EXTRACT

The fresh leaves were washed with distilled water and air dried for about 21days. The dried plant materials were pulverized into coarse powder using Thomas Willey Laboratory Mill, Model 4. About five hundred grams of the pulverized material were macerated in 95% ethanol and extracted for 7 days with 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)

PHARMACOLOGICAL ASSAYS

ANIMALS

The experiments were performed on adult albino rats of both sexes (150-200 g) and mice (25-30g). The animals were obtained from the Department of Zoology and Environmental Biology, University of Nigeria, Nsukka Enugu State, Nigeria. They were housed in groups of 5 per cage to acclamatize and were kept at room temperature of 20-25^{0}C on 12 h light/dark cycle with food and water administered *ad libitum*. Experiments were performed in accordance with the International Guide for the Care and use of Laboratory Animals.

ACUTE ORAL 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 – 30 g were divided into groups (n = 5). Animals were administered 10 mg/kg body weight of the extract up to the dose of 5000 mg/kg body weight and monitored for clinical signs and mortality.

DESIGN FOR DNFB – INDUCED PSORIASIS MODEL

The anti-psoriatic assay of the extract was done using the method described by Dong *et al* (2013)[14] with slight modifications. The dorsal skin of adult albino rats were

shaved using depilatory equipment before the experiment. Animals were divided into 5 groups (n = 5).

Group I: Served as vehicle control (acetone-olive oil was applied to the shaved abdomen).

Group II: Served as the negative control, induced without treatment.

Group III: Induced and received 200 mg/kg body weight of extract.

Group IV: Induced and received 400 mg/kg body weight of extract.

Group V: Served as the positive control, induced and received 0.5 mg/kg body weight of retinoic acid.

Induction was achieved by repeated application of 100 μL of 0.5% DNFB in (4:1) acetone-olive oil (vehicle) to the shaved abdomen on days 1, 2, 3, 5 and 7. On days 8, 9 and 10, animals were challenged by the application of 20 μL of 0.2% DNFB in a mixture of acetone and olive oil (4:1) to both sides of the ear. Treatment groups received 200 mg/kg, 400 mg/kg body weight of the extract orally for 14 days. The positive group was treated with retinoic acid 0.5 mg/kg body weight. Twenty-four hours post treatment, the animals were sacrificed and skin samples excised for histological examination.

HISTOLOGICAL ANALYSIS

Skin samples were excised from the rats and fixed with 10% formaldehyde, embedded in paraffin wax, routinely processed and then sectioned into 4 – μm thick slides. The skin sections were then stained with Hematoxylin-eosin and examined by light microscopy for the presence of inflamed cells[15]. Percent inhibition of epidermal thickness, ear thickness and ear weight was calculated according to the equation:

$$\% \text{ inhibition} = \frac{X_N - X_{DNFB}}{X_N - X_P} X100$$

Where X_N = Scores obtained from the disease model (negative control)

X_{DNFB} = Scores obtained from DNFB induced plus *H. madagascsriensis* treated animals

X_P = Vehicle treated (positive control)

STATISTICAL ANALYSIS

The results were statistically assessed using a one way analysis of variance (ANOVA) and the data presented as mean ± standard deviation (SD). Multiple *post-hoc* differences were checked by the Scheffe test. The p values equal or lower than 0.05 were considered statistically significant.

RESULTS

There were no signs of toxicity like convulsion, tremor, circling, depression, hypothermia, redness, erythema in skin. No death was recorded; hence, the extract is safe and has a wide range of effective dose.

The cutaneous application of DNFB on the rat's skin produced a pronounced erytherma. Repeated application showed a significant increase in epidermal thickness and reddening of the rat's skin as compared to the vehicle control group where the application of the vehicle (acetone – olive oil; 4:1) did not induce any skin differentiation. Epidermal thickness was measured to assess the severity of the epidermal hyperplasia induced by DNFB application. Epidermal thickness following histopathological examination revealed significant increase (two to three

times) in the DNFB group (Fig 1,Table 1) which was induced without any treatment when compared to the positive control. The inhibition effects of retinoic acid 0.5 mg/kg, *H. madagascariensis* leaf extract 200 mg/kg and 400 mg/kg showed an encouraging decrease in epidermal thickness by 61.21%, 60.33% and 74.63% respectively when compared to the two control groups (Table 1, 2 and Fig. 2).

Table 1: Inhibition effects of *H. madagascariensis* leaf 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	Leave extract 200 mg/kg	Leave extract 400 mg/kg	Positive control Retinoic acid 0.5 mg/kg
Epithermal thickness (μm)	34.00±6.00	111.33±11.72	64.67±5.57**	53.62±5.57#	64.00±13.49
Ear weight(mg)	70.60±5.22	248.66±1.76	130.76±0.29*	112.42±0.6#	138.42±7.76
Ear thickness (μm)	0.59±0.05	1.22±0.06	0.76±0.20*	0.62±0.03#	0.71±0.08

Data presented as mean ± SD (n = 5). *P<0.05 compared to the positive control, **P<0.05 compared to positive control. # - significantly lower than the negative control, (P<0.05)

Table 2: Percentage (%) inhibition of DNFB – Induced epidermal thickness, ear weight and ear thickness by H. madagascariensis leave extract.

Parameter	Positive control Retinoic acid (0.5 mg/kg)	Extract (200 mg/kg)	Extract (400 mg/kg)
Epithermal thickness (%)	61.21	60.33	74.63
Ear weight (%)	56.27	66.21	76.51
Ear thickness (%)	33.33	49.46	64.52

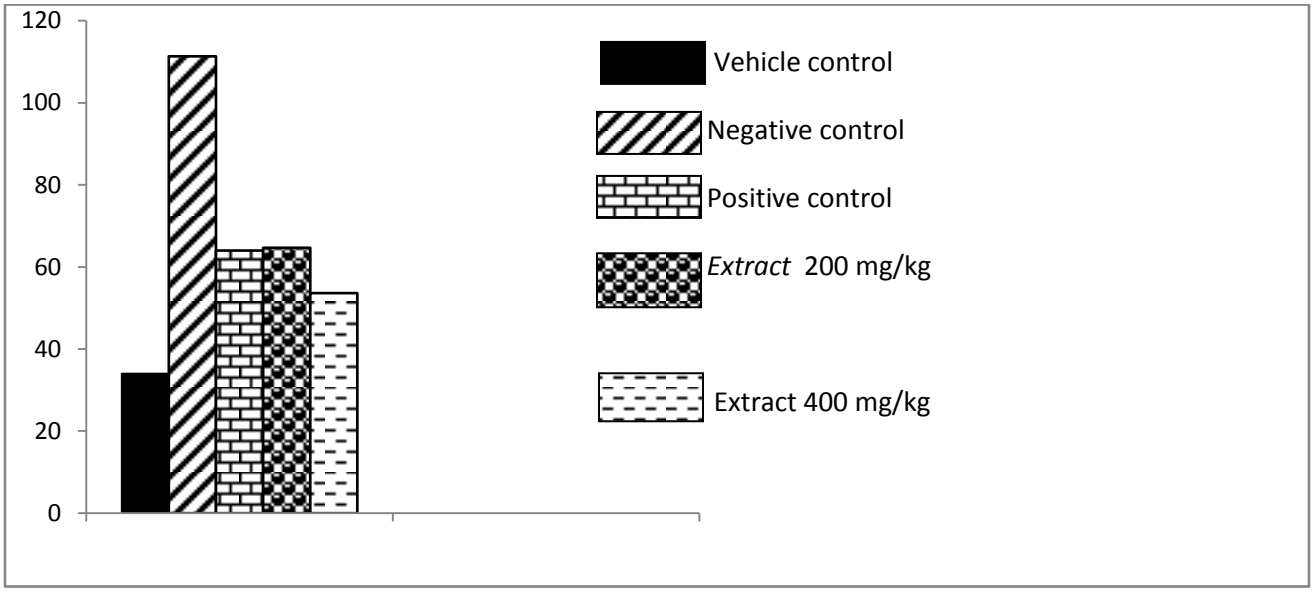

Fig 2: *Reduction of DNFB – induced epidermal thickness (µm) in rats by H. madagascariensis leave ethanol extract and retinoic acid.*

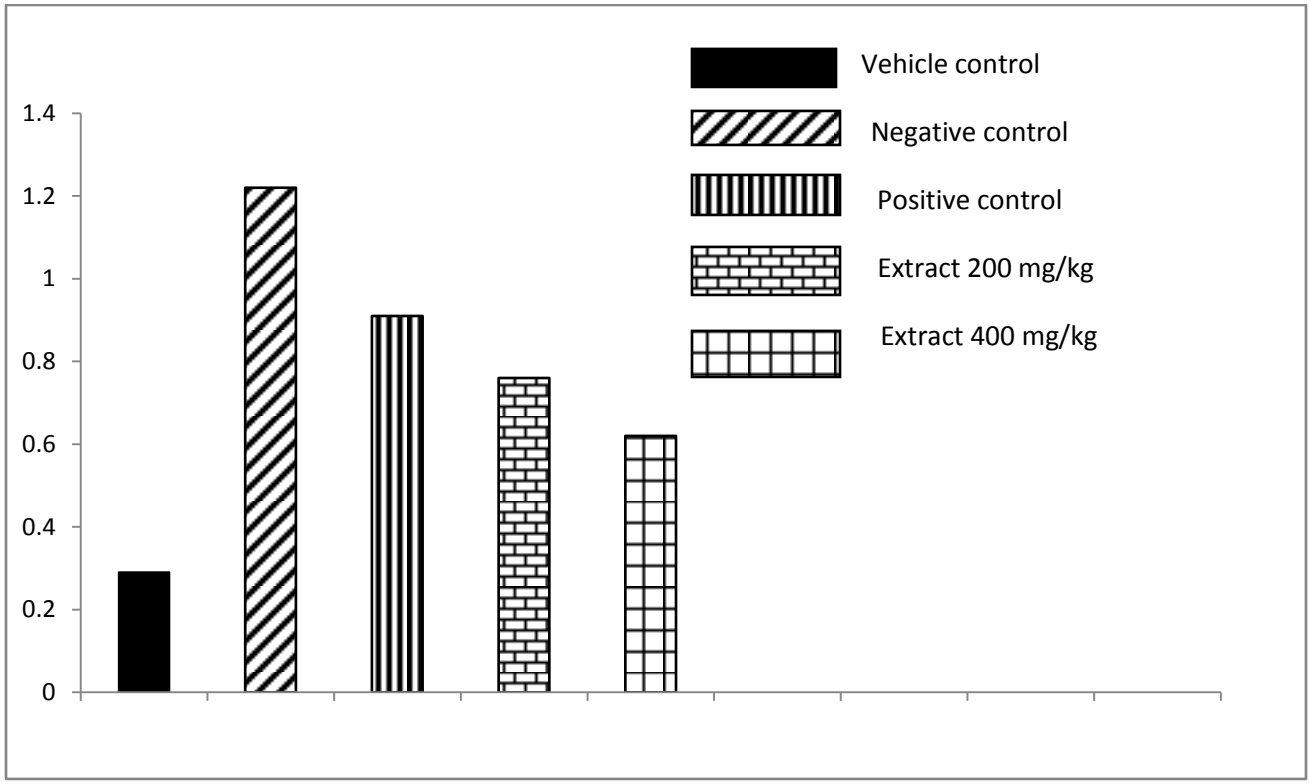

Fig 3: *Reduction of DNFB – induced ear thickness (µm) in Rats by H. madagascariensis leave ethanol extract and retinoic acid.*

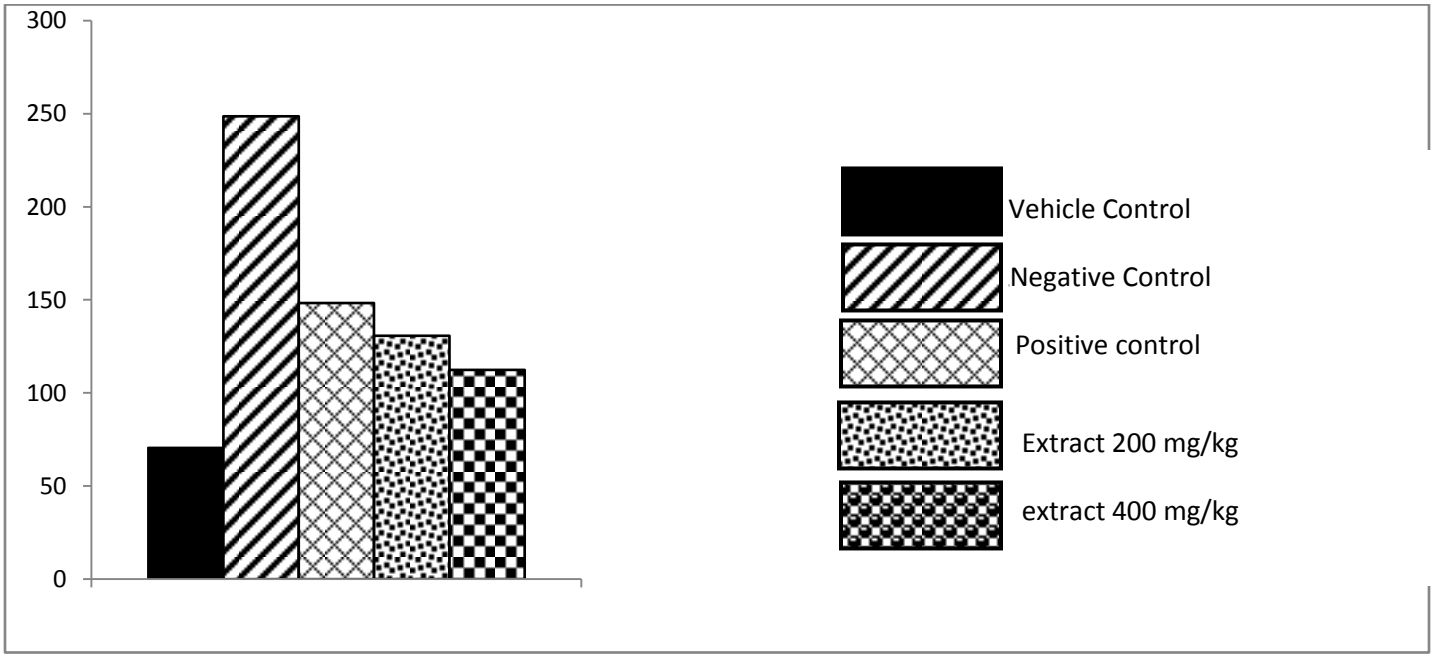

Fig 4*: Reduction of DNFB – induced ear weight (mg) by H. madagascariensisleave ethanol extract and retinoic acid.*

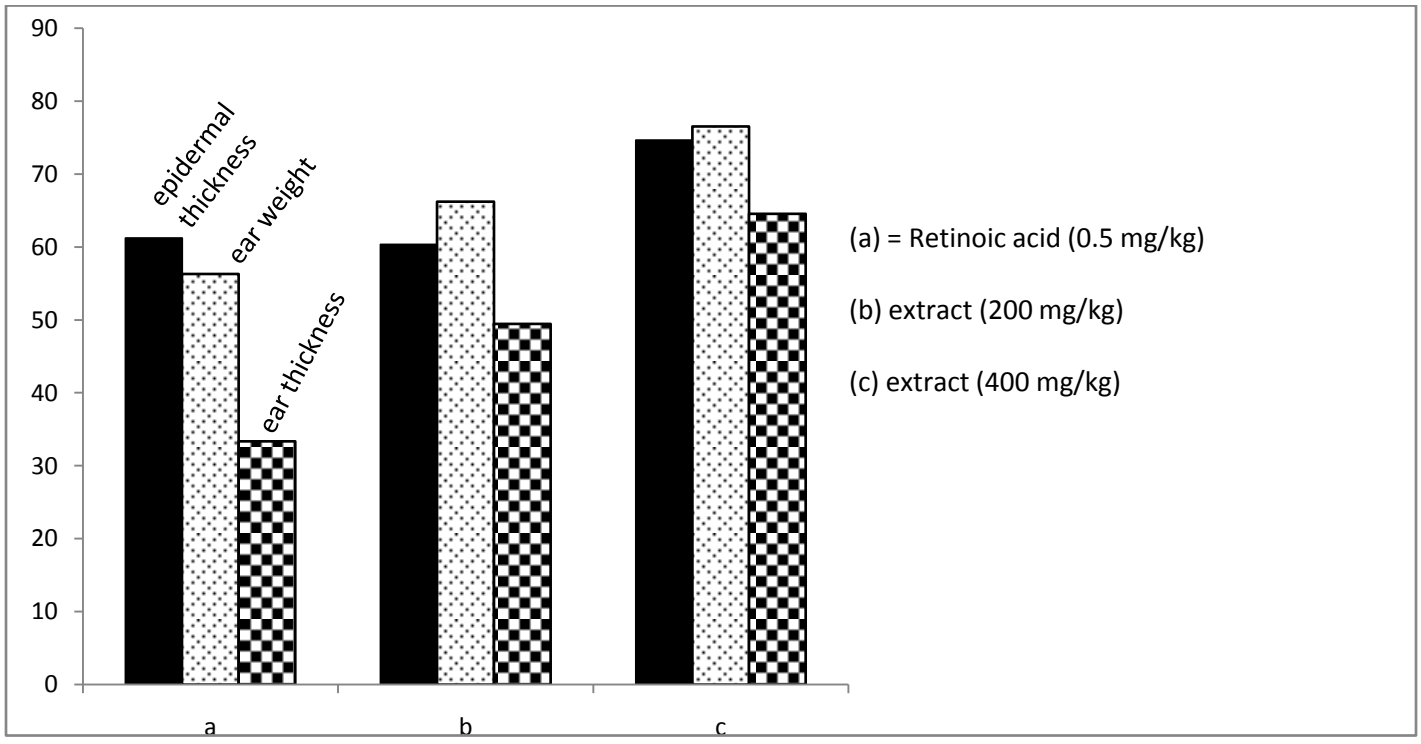

Fig 5: *Percent (%) inhibition of DNFB – induced epidermal thickness (µm), ear weight (mg) and ear thickness (µm) by H. madagascariensis leave ethanol extract and retinoic acid.*

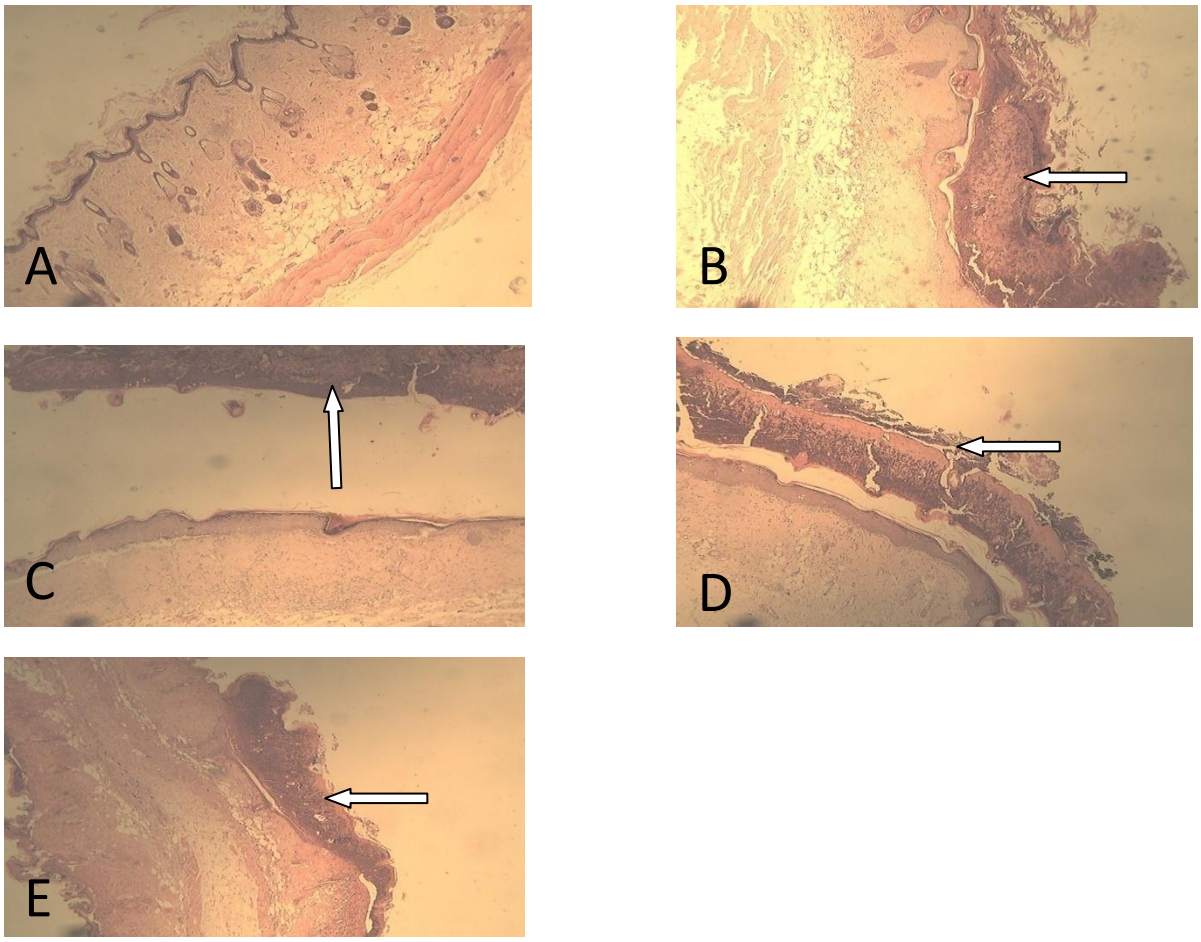

Figure 1*: Photomicrograph of the skin showing the histopathological features of DFNB induced psoriasis in rats treated with different doses of the extract and retinoic acid; A = vehicle control, B = negative control, C = H. madagascariensis, 400 mg/kg, D = retinoic acid, 0.5 mg/kg, E = H. madagascariensis, 200 mg/kg.*

Table 1 and 2 as well showed a suppressive rate of both the ear thickness and weight as compared to the control groups. Gross macroscopic examination revealed a relative increase in ear weight and thickness in the disease model as compared to the positive control. Oral treatment with *H. madagascariensis* ethanol leaf extract potently reduced DNFB-induced oedema/or weight by 76.51%, while Ear thickness was as well reduced by 64.52%. Animals treated with retinoic acid at the concentration of 0.5 mg/kg

body weight decrease ear weight and thickness by 56.29% and 33.33% respectively (Table 2).

DISCUSSIONS

An anti-psoriatic drug that targets the epidermis is a compound that restores the skin homeostasis by suppressing keratinocyte hyper proliferation, abnormal differentiation or both[16]. Psoriasis is a chronic inflammatory skin disease associated with cutaneous hyper reactivity to environmental triggers and skin inflammatory responses driven by cytokines expression.

For the first time, we evaluate and report the inhibitory properties of *H. madagascariensis* ethanol leaf extract against chronic DNFB – induced psoriasis in rat. Psoriatic lesion was induced in shaved abdomen and ear of albino rats by repeated topical application of 0.5% DNFB. This produced a prominent epidermal hyperplasia and increased ear weight and thickness. In this study, oral treatment with *H. madagascariensis* ethanol leaf extract significantly reduced epidermal thickness, ear thickness and ear weight.

The result of our study agrees with literature reports for the use of *H. madagascariensis* leaf (unopened bud) for the treatment of skin disorders, and wound infections[10]. Researchers who have used DNFB-induced model[10, 13, 14] reports that its repeated application significantly raised epidermal thickness to twice or thrice 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. The leaf ethanol extract of *H. madagascariensis* showed 74.63% inhibition of epidermal thickness at 400 mg/kg and was revealed to have more activity than the standard drug (retinoic acid 0.5 mg/kg) which showed 61.21% effectiveness. Vijayalakshmi and Madhira (2014) in their work showed that 400 mg/kg of their ethanol extract of *C. tora* leaves reduced epidermal thickness in rats from 94.86 (μm) (positive control) to 38.52 (μm) (61.03%) using the photo-induced model, to be more effective than the standard drug (retinoic acid 0.5 mg/kg body weight). Histopathological and gross macroscopic examination as well showed good

inhibitory effects of *H. madagascariensis* on ear thickness and weight. The main finding of the present paper is that ethanol extract of *H. madagascariensis* leaf inhibited DNFB-induced psoriasis by 74.63% at 400 mg/kg compared to 61.21% by the control group[16].

CONCLUSION

We recommend further purification of the extract to actually ascertain the fraction or isolated compound responsible for the reported activity.

CONFLICT OF INTERCEPTS

The authors declare that they have no conflicts of interest

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