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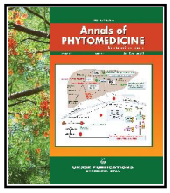

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Assessment of antipsoriatic effect of ethanol extract from *Decalepis hamiltonii* Wight & Arn. roots in a dinitrofluorobenzene induced psoriasis rat model

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Abstract

This study aimed to explore the therapeutic potential of the ethanolic extract of *Decalepis hamiltonii* Wight & Arn. roots (EEDHR) in the context of psoriasis, leveraging a 2,4-dinitrofluorobenzene (DNFB)-induced psoriasis model in rats. The primary objectives were to assess the antipsoriatic effects of EEDHR through anti-inflammatory, hematological, and histological evaluations. EEDHR were historically used by traditional Indian tribes in the Western Ghats region for various ailments. In this study, we prepared the ethanolic extract of EEDHR and administered it to rats in a DNFB-induced psoriasis model. Anti-inflammatory effects were assessed through measurements of epidermal thickness, while hematological examinations, including total white blood cell count (TWBC), were conducted to explore potential immunosuppressive activity. Histopathological analysis was employed to evaluate cellular responses and collagen fiber synthesis. Our study revealed a substantial reduction in epidermal thickness (90.35%) in rats treated with EEDHR. This significant decrease indicated a promising antipsoriatic effect. Furthermore, the observed reduction in TWBC suggested potential immunosuppressive activity, likely attributed to the suppression of proinflammatory cytokine production. Histopathological analysis indicated the presence of normal keratinocytes, epithelialization, and substantial collagen fiber synthesis, highlighting the extract's capability to stimulate healing responses. This research unveils the anti-inflammatory and immunosuppressive properties of EEDHR, shedding light on its potential for mitigating drug-induced psoriasis in rats. The remarkable reduction in epidermal thickness and positive histopathological findings underscore the novel therapeutic attributes of EEDHR in managing psoriasis.

These findings not only provide valuable insights into the pharmacological properties of EEDHR but also open avenues for further exploration and potential applications in the treatment of psoriasis.

1. Introduction

In Western countries, 2-3% of Caucasian people suffer with psoriasis, a chronic inflammatory illness mediated by the immune system. Psoriasis treatment can help clear the skin, but it cannot cure the condition. Topical therapy is the most generally used treatment for restricted (mild) illness, with phototherapy added in patients that do not respond well. It is advised to use either systemic therapy alone, in conjunction with phototherapy, or systemic therapy alone for moderate to severe psoriasis. Current guidelines outline the strength of the evidence supporting the effectiveness of the therapies already in use and suggest using them in routine practice (Mrowietz *et al*., 2011). When non-steroidal anti-inflammatory medicines (NSAIDs) are used to treat inflammation and discomfort, some of the main side effects include gastritis, renal failure, and retention of salt and water (Rainsford, 2007). The combined problems of synthetic medication resistance and side effects need an urgent search for novel and potent therapeutic plant molecules. We must investigate efficient alternative medicine in order to prevent the negative consequences of synthetic pharmaceuticals. Because they are readily available and have few to no negative effects, traditional herbs have been used for a very long time and are considered trustworthy (Cathrine and Nagarajan, 2011).

Phytochemicals function through a range of signaling pathways either jointly or independently from one another. Phytochemicals such as flavonoids, fatty acids, and naturally occurring polyphenols have been demonstrated to be efficacious in the prevention and treatment of various ailments. Their antioxidative and free radical scavenging abilities enable them to regulate the inflammatory response of certain cells, including mast cells, neutrophils, lymphocytes, and macrophages (Alolga *et al*., 2015). Snehal *et al*. (2023) have also displayed the immunomodulatory potential of *E. herbacea* Lind. tubers extract. Ethanolic and methanolic extracts of *D. candolleana* parts exhibited more antioxidant properties and anti-inflammatory due to the presence of active biochemical compounds such as phenols, glycosides, steroids, flavonoids, *etc.* (Anvitha *et al*., 2023).

Persistent inflammation leads to uncontrolled keratinocyte proliferation and poor differentiation, which is indication of psoriasis. The histology of the psoriatic plaque shows inflammatory infiltrates composed of neutrophils, T cells, macrophages, and dermal dendritic cells, accompanied by acanthosis, or epidermal hyperplasia (Rendon *et al*., 2019). Some drugs are thought to be psoriasis' alleged causative factors, as well as systemic corticosteroid withdrawal and oxidative

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stress (Zhou *et al*., 2009). Free radical generation causes DNA damage that can be alleviated by the presence of antioxidants (Pamela *et al*., 2018). Because of their capacity to alter a number of biochemical processes, fatty acids are specifically considered among anti-inflammatory drugs and may be crucial for the treatment of autoimmune illnesses like psoriasis (Geetha, 2014). Psoriasis sufferers' skin has a high leukotriene concentration because of their disordered arachidonic acid metabolism (Bowcock, 2005). White blood cell infiltration brought on by chemotactic and pro-inflammatory factors particularly cytokines and lipid mediators are the first step toward the development of psoriasis (Mayser *et al*., 2008). Complementary and alternative therapies are used by around 69% of patients with dermatological diseases; therefore, they may show promise in the treatment of psoriasis (Dhanabal *et al*., 2012). Indian traditional medicine known as Ayurveda has made use of *D. hamiltonii* roots., to promote appetite, treat skin conditions, diaphoretic, hemorrhoids, rheumatism, asthma, bronchitis, somatic and antiviral, and as a general tonic. The ancient tribes of India's Western Ghats used *D. hamiltonii* roots as an aqueous extract and decoction to treat ulcers, fever, inflammation, and other ailments (Reddy and Murthy, 2013).

Ashalatha *et al*. (2010) have reported that the chemicals that were extracted from the roots of *D. hamiltonii* showed anti-inflammatory properties by inhibiting the mRNA specific to TNF-α and IL-2 and increasing the mRNA synthesis of IL-10. Scientific evidence supporting the antipsoriatic and immuno-modulatory effects of *D. hamiltonii* roots is currently lacking or non-existent. Considering this context, the research aimed to assess the therapeutic impact of an ethanolic extract derived from *D. hamiltonii* roots in a rat model with psoriasis induced by dinitrofluorobenzene.

2. Materials and Methods

2.1 Drug

The drug dinitrofluorobenzene, carrageenan and xylene were analytical grade and procured from Sigma Aldrich, Mumbai, India.

2.2 Collection of plant

Decalepis hamiltonii Wight & Arn was collected from the surrounding region, cleaned with tap water, cut into small slices, and dried for two weeks at room temperature (28 ± 1°C). The taxonomic identification (SMPU/CARI/BNG/2022-23/8781, dated: 24.02.2023) of *D. hamiltonii* roots attributing it to the Asclepidaceae family, was performed by a taxonomist (Dr.V. Rama Rao) at the Central Ayurveda Research Institute, Thalaghattapura Post, Bengaluru-560109. Ethanolic extract of *D. hamiltonii* roots (EEDHR) was obtained as dry powder from Green Chem Pvt Ltd, Bengaluru, Karnataka, India.

2.3 Phytochemical assessment of ethanol extract of *D. hamiltonii* roots (EEDHR)

Preliminary phytochemical analysis was undertaken as per definitive methods for the comparative assimilations of phytoconstituents.

- **Alkaloids**: Alkaloids were detected using the Dragendorff's reagent. Briefly, a few drops of the reagent were added to the plant extract, resulting in the formation of orange-brown precipitates.
- **Flavonoids**: The presence of flavonoids was determined by treating the extract with magnesium and hydrochloric acid. The development of an intense color indicated the presence of flavonoids.
- **Tannins:** Tannins were identified using ferric chloride. The addition of ferric chloride to the extract resulted in a blue-black or greenish-black color, indicating the presence of tannins.
- **Saponins:** Saponins were detected by frothing test. The extract was vigorously shaken with water, and the persistence of froth indicated the presence of saponins.
- **Terpenoids:** Terpenoids were identified using the Salkowski test. The plant extract was treated with chloroform and concentrated sulfuric acid, resulting in the development of a reddish-brown color, confirming the presence of terpenoids.
- **Glycosides:** The presence of glycosides was determined using the Bontrager's test. The extract was treated with glacial acetic acid and sulfuric acid, producing a pink or red color, indicating the presence of glycosides.
- **Carbohydrates:** Carbohydrates were detected using Benedict's test. The plant extract was mixed with Benedict's reagent and heated, leading to the formation of a colored precipitate.

2.4 Approval for study

As per the guidelines of the committee for the purpose of control and supervision of experimental animals (CPCSEA), below experiment was carried out with reference number KCP/IAEC/PCOL/111/2022, approved by Institutional Animal Ethics Committee (IAEC) of Krupanidhi College of Pharmacy, Bangalore, India.

2.5 Animal study model

We used albino Wister rats that weighed between 150 and 200 g and mice weighing between 22 to 25 g. The rats and mice were handled as per the CPCSEA guidelines and regulation. Under normal circumstances, the animals are housed at the Krupanidhi College of Pharmacy in the college's animal facility. Maintaining a constant temperature (23 ± 2°C) and humidity; providing 12 h of light (7:00 am - 7:00 pm). The study protocol was carried out after obtaining the approval from the Institutional Animal Ethics Committee (IAEC) of Krupanidhi College of Pharmacy, Bangalore, Karnataka, India. Dinitrofluorobenzene induced psoriasis model was performed to evaluate the antipsoriatic property of EEDHR.

2.6 Segregation of animals

2.6.1 Acute toxicity study in mice

Both sex of Albino mice were divided into five groups. Animals were delivered between 10 to 5000 mg/kg of EEDHR and observed for 24 h as per OECD 425 guidelines (Saleem *et al*., 2017).

2.6.2 Treatment protocol

Group 1: Vehicle control (The shaved abdomen was covered with olive oil and acetone).

Group 2: Negative control induced with dinitrofluorobenzene (topical application).

Group 3: Psoriasis rats treated with low dose of EEDHR (200 mg/kg *b.w,* orally).

Group 4: Psoriasis rats treated with high dose of EEDHR (400 mg/kg *b.w,* orally).

Group 5: Positive control (induced and received standard drug retinoic acid 0.5 mg/kg *b.w*, topical application).

As per regulation and guidelines, each group consists of 5 animals kept in cage on regular diet.

2.7 Psoriasis area severity index (PASI) scoring evaluation

The degree of inflammation in the back area was rated using the psoriasis area severity index scoring system. Erythema/redness and scaling were evaluated independently on a 0-4 scale where 0 represents the absence of a feature, 1 indicates a minimal presence, 2 signifies a moderate level, 3 denotes a significant presence, and 4 suggests an extremely significant presence. As a gauge of the severity of psoriasis, the sum of the aforementioned independent scores is known as the PASI cumulative score (Flutter and Nestle, 2013). Epidermal and ear skin thickness were precisely measured using Vernier calipers. The calipers were set parallel to the skin, ensuring exclusive measurement of the skin without including the ear cartilage. To achieve accuracy, the calipers were gently applied to the back skin and the ear, and measurements were recorded in micrometers (μm) to the nearest decimal place. Equation (1) was used to calculate the % inhibitions for above measurements. The following outcomes were noted:

$$\% \text{ inhibition} = X_N - X_{DNFB} / X_N - X_P \times 100 \quad (1)$$

where, X_N = The results of the negative control's scores

X_{DNFB} = Scores from animals treated with roots extract and those stimulated with DNFB.

X_P = sum of the vehicle control's scores.

2.8 Evaluation of antipsoriatic activity

Before the trial began, healthy adult albino rats weighing between 150 and 200 g had their abdominal skin shaved using depilatory equipment. Throughout the course of five days, on days 1, 2, 3, 5, and 7, 100 μl of a solution of 0.5 per cent dinitrofluorobenzene in a 4:1 mixture of acetone and olive oil was administered frequently to the shaved abdomen to create the ideal state. The animals were challenged three days following the induction procedure by topically applying 50 μl of a 0.2% acetone-olive oil dinitrofluorobenzene solution (again in a 4:1 ratio) to both ears over the course of three days on days 9, 10, and 11. The ears remained unshaven. During the trial, animals were divided into groups of five and given oral doses of the extract or the pure medication at the appropriate concentration for 14 days after either induction or challenge (Dong *et al*., 2013)

2.9 Immuno-modulatory assessment

This action of EEDHR was assessed using two models: A haematological assay that employed standard pharmacological procedures, and an anti-inflammatory paradigm (oedema generated by carrageenan and xylene).

2.9.1 Carrageenan-induced paw oedema

Oedema was produced using a protocol that had been explained previously (Winter *et al*., 1962), with few modifications. Each animal's right hind paw received a 0.1 ml injection of a 1% carrageenan suspension subcutaneously, and the paw volumes were measured 1 to 5 h after administration. Paw volume baseline measurements were taken at zero hours before injecting carrageenan, and then again at 1 to 5 h following treatment. The measurement of oedema volume was deemed to be the increase in paw swelling compared to the animals in the normal group. The following algorithm [Equation (2)] was then used to determine the % inhibition of oedema.

$$\% \text{ inhibition} = [(Vt - V_0) \text{ normal} - (Vt - V_0) \text{ treated}]/(Vt - V_0) \text{ normal} \times 100 \quad (2)$$

where, V_0= paw volume at 0 hr., Vt= paw volume at 1 to 5 h.

2.9.2 Xylene-induced ear oedema

Approximately 60 min later following the administration of the specified dosage to each animal, 20 μl of xylene was administered to both surfaces (anterior and posterior) of right ear, while the left ear neither received induction nor medical intervention. Subsequently, after an additional 60 min, the animals were anesthetized, and samples were collected from both ears using a 5 mm diameter punch biopsy tool. By comparing the weight variations between the biopsies obtained from the right and left ears of the same animal, the degree of ear oedema was ascertained.

2.10 Haematological analysis

Assessments of hemoglobin concentration, packed cell volume, distinct counts of leucocytes, red blood cells, and white blood cells were conducted in accordance with established and recognized methods and procedures. This procedure was carried out following the guidelines provided by Bancroft and Gamble's instructions (Bancroft and Gamble, 2008). Skin samples affected by the conditions were randomly selected from different groups at the end of each trial. These samples were preserved in 10% normal saline, dehydrated by adding progressively more ethanol to the mixture, and then cleaned for an entire night in chloroform. Subsequently, the tissues were infiltrated with and embedded in liquid paraffin wax. Subsequently, the paraffin blocks were divided into pieces of 5 and 6 μm. The sections were first deparaffinized in xylene, rehydrated in water, then stained with hematoxylin and eosin (H and E) in order to get them ready for light microscopy. A motion camera that was connected to a light microscope was used to take photomicrographs of the sections.

2.11 Statistical analysis

Dunnett's test was conducted after one-way analysis of variance (ANOVA) was used to assess the statistical comparison. mean ± SEM was used to report values, and a significance threshold of $p<0.05$ was employed to indicate statistical significance.

3. Results

3.1 Preliminary phytochemical screening of *D. hamiltonii* roots extract

A qualitative phytochemical screening on EEDHR indicates the existence of steroids, alkaloids, flavonoids, tannins, terpenoids, and cardiac glycosides.

3.2 Acute toxicity results

A high level of safety was observed in the raw extract, as indicated by the findings of the acute toxicity investigation. No animal deaths were observed, even when the dose was increased to 2000 ppm (parts per million).

3.3 Psoriasis area severity index (PASI) score evaluation

It has been shown that repeatedly applying DNFB to rats' shaved skin can successfully elicit symptoms similar to psoriasis. Redness or erythema was visible after the first treatment, and these symptoms got worse after the second and third applications (Figure 1).

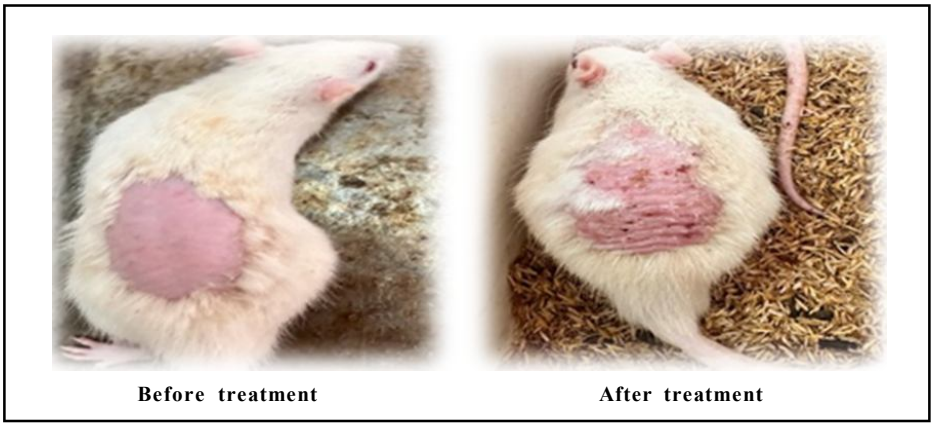

Figure 1: Image of psoriasis caused by DNFB's topical application.

Table 1: Effect of EEDHR on DNFB psoriasis induction animals' erythema and scaling severity scores

	Days of treatment	Negative control	Positive control (RA 0.5%)	Low dose of EEDHR (200 mg/kg)	High dose of EEDHR (400 mg/kg)
Redness/Erythema	0	4 ± 0	4 ± 0	4 ± 0	4 ± 0
	4	4 ± 0	3.2 ± 0.45***	4 ± 0	4 ± 0
	7	4 ± 0	2.8 ± 0.84**	4 ± 0	3.4 ± 0.55
	10	4 ± 0	2.4 ± 1.34*	3.4 ± 0.55	2.8 ± 0.84
	13	3.8 ± 0.45	1.8 ± 0.84****	2 ± 0***	1 ± 0****
	16	3.2 ± 0.45	1 ± 1.22***	1 ± 0.45***	1 ± 0****
Scaling	0	4 ± 0	4 ± 0	4 ± 0	4 ± 0
	4	4 ± 0	3.6 ± 0.55	4 ± 0	4 ± 0
	7	4 ± 0	3.6 ± 1.09	4 ± 0	4 ± 0
	10	4 ± 0	2.6 ± 1.52	4 ± 0	3.6 ± 0.9
	13	4 ± 0	2 ± 1***	2.6 ± 0.55*	2 ± 0.7***
	16	3.6 ± 0.55	1.4 ± 1.52***	1.2 ± 0.45***	1 ± 0***

RA: retinoic acid, with n = 6, all values are presented as mean ± SEM.

One-way ANOVA is used for analysis, and Dunnett's test is used to determine significance (*p<0.05, **p<0.01, ***p<0.001, and ****p<0.0001) when compared to the control group

Every three days over the course of the therapy, the degree of psoriasis was evaluated macroscopically (Table 1). Remarkably, the rats assigned to receive the EEDHR or retinoic acid saw a progressive decline in the psoriasis severity index. Compared to the negative control group, an impressive reduction in the severity index was noted for redness and erythema after just three days for the standard group (retinoic acid at 0.5%; p<0.001), and after 12 days for the plant extract (p<0.001 for both 200 mg/kg and 400 mg/kg.). In terms of scale, the severity score dropped following a 12-day course of treatment in a statistically significant way in the groups who had the EEDHR or retinoic acid. The EEDHR or retinoic acid treatment was found to consistently reduce the overall severity score, which considers both redness/erythema and scaling (Table 1). At the conclusion of the study period, the rats in the standard group (0.5%) had a cumulative score of 2.4, whereas the rats in the EEDHR treated group had a score of 2.2 (Figure 2).

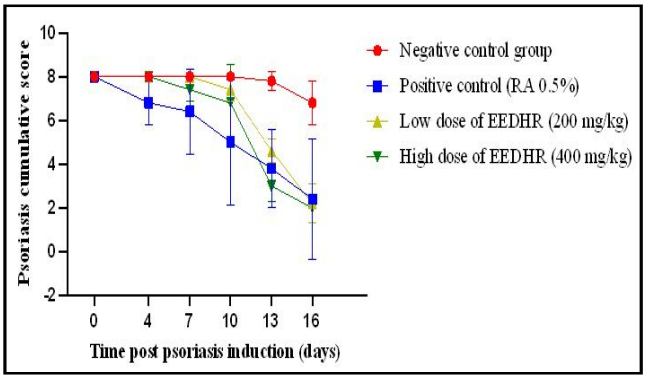

Figure 2: Effect of EEDHR on psoriasis cumulative score values expressed in mean ± SEM.

3.4 Ear thickness and epidermal skin thickness

The antipsoriatic activity was evaluated by measuring the ability to reduce induced epidermal thickness (EDT), ear weight (EW), and ear thickness (ET). Figure 3 presents the results of the % acitivty of extract at different doses, which demonstrated significant antipsoriatic effects. More specifically, the extract showed a remarkable 90.35% reduction in ET at a dose of 400 mg/kg body weight when compared to the positive control (retinoic acid) that exhibited only a reduction of 60.11%.

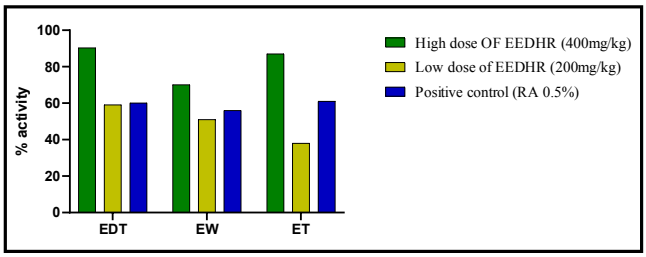

Figure 3: % activity of EEDHR.

3.5 Carrageenan-induced paw oedema

The anti-inflammatory test employing varying dosages of EEDHR yielded paw sizes of 1.31 ± 0.06 for both the 200 mg/kg and 400 mg/kg body weight doses (Figure 4); however, there was no significant difference ($p<0.05$) in the paw sizes. The % inhibition of paw edema varied from 32.87% to 43.78% and 42.44% to 54.81% at 200 mg/kg and 400 mg/kg of the ethanol extract, respectively. These results paled in comparison to the range of 29.93% to 71.65% for the percentage inhibition attained by indomethacin at 10 mg/kg body weight (Figure 4). But, when time was taken into account, the outcomes showed that the 400 mg/kg EEDHR was more efficacious than indomethacin between 1 and 3 h after carrageenan induction. Specifically, for EEDHR, the % inhibitions per hour were 42.44%, 47.20%, and 50.10% for the first, second, and third hours, respectively. At similar time intervals, indomethacin demonstrated percentage inhibitions of 29.93%, 43.54%, and 47.86%.

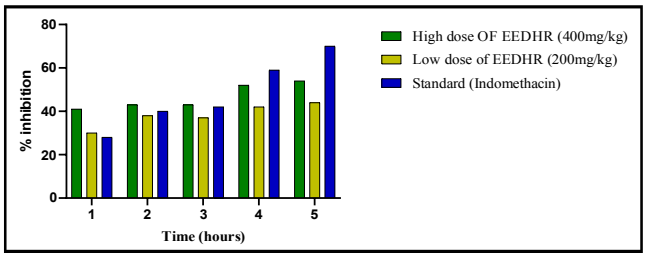

Figure 4: % inhibition rate of EEDHR and indomethacin-induced rat paw oedema.

3.6 Xylene-induced ear oedema

The extract at doses of 200 and 400 mg/kg demonstrated substantial % inhibitions of 62.47 and 69.70% on xylene-induced ear oedema respectively (Table 2). The inhibitory impact at 400 mg/kg was comparable to that of the prescription drug dexamethasone, with an inhibition of 74.56%.

Table 2: Reduction of xylene-induced ear oedema by EEDHR and standard drug (dexamethasone 10 mg/kg)

Treatment groups	Right ear's weight (g)	Left ear's weight (g)	Difference (g)	% inhibition
Negative control (H_2O 5 ml/kg)	0.026 ± 0.002	0.017 ± 0.001	0.009 ± 0.001	-
Low dose of EEDHR (200 mg/kg)	0.013 ± 0.004	0.005 ± 0.003	0.007 ± 0.001**	62.47
High dose of EEDHR (400 mg/kg)	0.011 ± 0.006	0.005 ± 0.002	0.006 ± 0.004***	69.70
Standard (dexamethasone 10 mg/kg)	0.008 ± 0.006	0.003 ± 0.005	0.005 ± 0.001****	74.56

All the values are presented as mean ± SEM, with n = 6.

One-way ANOVA was employed for analysis, and Dunnett's test was used to determine the significance levels for comparisons between the treated groups and the negative control group (*$p<0.05$, **$p<0.01$, ***$p<0.001$ and ****$p<0.0001$).

3.7 Haematogical analysis

One important diagnostic indicator is the analysis of hemoglobin status. The normal range of these parameters can be changed, either positively or negatively, by the oral intake of chemicals or medications. Table 3 illustrates how various dosages of EEDHR and their influence on hematological parameters over the course of a 14-day period.

The findings of this study demonstrated that while TWBC decreased, RBC, PCV, and Hb increased. This decline, suggests that the plant had an immunosuppressive impact. The altered blood parameters may be caused by modifications in cellular integrity, membrane permeability, metabolism, or even exposure to toxic compounds. These changes imply that the plant extract contains phytochemicals that may either increase or decrease the generation of leucocytes or erythrocytes.

Table 3: Effects of a 14-day therapy with varying dosages of EEDHR on rats' hematological parameters

Groups	Haematogical analysis					
	PCV (%)		Hb (g/dl)		RBC (x 10^3 cell/mm^3)	
	Initial blood count	Final blood count	Initial blood count	Final blood count	Initial blood count	Final blood count
Negative control	60.67 ± 4.71	62.20 ± 0.72	17.60 ± 0.33	17.08 ± 0.23	6.08 ± 0.27	6.01 ± 0.1
Low dose of EEDHR (200 mg/kg)	51.00 ± 4.32	62.75 ± 1.54*	13.66 ± 0.45	16.06 ± 0.14*	5.42 ± 0.35	6.20 ± 0.05*
High dose of EEDHR (400 mg/kg)	47.00 ± 1.01	61.82 ± 2.39*	13.66 ± 0.17	18.77 ± 0.24*	5.27 ± 0.05	6.77 ± 0.07*

Groups	Haematogical analysis					
	N (%)		TWBC (cell/mm^3)		L (%)	
	Initial blood count	Final blood count	Initial blood count	Final blood count	Initial blood count	Final blood count
Negative control	63.33 ± 9.07	38.67 ± 9.07	5611.43 ± 121.98	5982.33 ± 92.11	38.67 ± 0.97	41.33 ± 0.52
Low dose of EEDHR (200 mg/kg)	59.67 ± 1.53	69.0 ± 1.00*	5716.32 ± 103.0	5542.33 ± 92.79**	36.67 ± 1.31	32.67 ± 0.51**
High dose of EEDHR (400 mg/kg)	65.33±2.31	69.33 ± 5.51**	5535.76 ± 186.00	5422.23 ± 103.52***	41.33 ± 1.57	33.00 ± 0.50***

All values are presented as mean ± SEM, with n = 6. One-way ANOVA was used for analysis, and Dunnett's test was employed to determine the significance levels when comparing all treatment groups to the negative control. *p<0.05, **p<0.01 and ***p<0.001 were used in this case.

3.8 Histopathological studies

Healing response in skin were significantly noticed in standard, low and high dose treated groups. Normal keratinocytes resulted in epithelialization and ECM differentiation with extensive collagen fibers as a result of healing response (Figure 5).

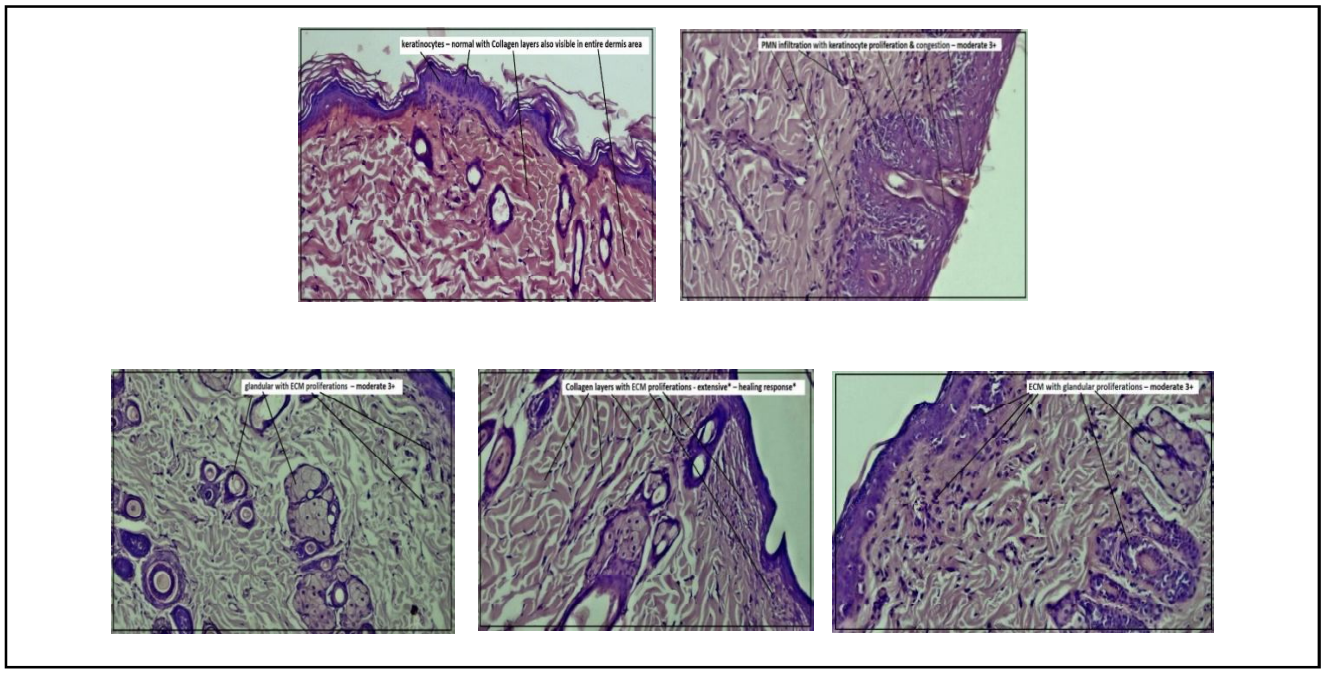

Figure 5: Histopathological studies of skin. a) Vehicle control rat skin showing keratinocytes – normal with Collagen layers also visible in entire dermis area (100×). b) DNFB induced rat skin showing congestion with keratinocyte proliferation and PMN infiltration) – moderate 3+ (100×). c) DNFB + Low dose of EEDRH treated rat skin showing ECM with glandular proliferations – moderate 3+ – NAD+ (100×) – healing response. d) DNFB + High dose of EEDRH treated rat skin showing ECM with Collagen layers proliferations – extensive* (100×) – healing response e) DNFB + Retinoic acid treated rat skin showing ECM with glandular proliferations – NAD+ (100×) – healing response*– moderate 3+.

4. Discussion

Psoriasis is characterized by persistent inflammation due to infiltration of inflammatory mediators (Rendon and Schakel, 2019) and high content of leukotrienes are observed in these patients due to disorganized metabolism of arachidonic acid (Bowcock, 2005). NSAIDs, or non-steroidal anti-inflammatory drugs, are used to treat inflammation, fever, and pain, both acute and chronic but leads to kidney problems, peptic ulcers, as well as retention of salt and water. Due to these reasons, there is an immediate need for a novel and potent therapeutic alternative such as phytochemicals. Numerous medical conditions are treated with biologically active phytochemicals, such as fatty acids, alkaloids, flavonoids, and other naturally occurring polyphenols. These phytochemicals' antioxidative and free radical scavenging abilities regulate the inflammatory processes of mast cells, lymphocytes, macrophages, and neutrophils (Raphael *et al.*, 2015).

Medicinal plants are widely used by traditional healers and are believed to be safe for human health, to cure variety of illnesses, including psoriasis. In the past, Indian tribes in India's Western Ghats used to cure inflammation, fever, ulcers, and other ailments with both the decoction and aqueous extract of *D. hamiltonii* roots. Based on scientific studies (Reddy and Murthy, *et al.*, 2013),

D. hamiltonii roots appear to have enormous biological potential. Several phytochemicals were extracted from *D. hamiltonii* roots and have shown a range of pharmacological activities. The aim of this research is to assess *D. hamiltonii* roots effectiveness in treating DNFB-induced psoriasis (Bancroft and Gamble, 2008) as there is a comprehensive data published on phytochemistry and other biological characteristics that support the role of this herb in treating animal model-induced psoriasis.

In an acute toxicity trial, the crude extract demonstrated high safety; no deaths were reported, even at a level of 2000 parts per million. The negative control showed a noticeable increase in epidermal thickness, which was supported by the histopathological examination. Thus, psoriasis induced by DNFB was accompanied by a substantial infiltration of inflammatory cells, hyperplasia of the epidermis, and enhanced skin oedema. Reducing ear thickness (ET) and epidermal thickness (EDT) caused by psoriasis served as a proxy for antipsoriatic activity. In the present investigation, the oral dose of 200 and 400 mg/kg body weight of *D. hamiltonii* roots extract proved beneficial in reducing psoriatic symptoms, specifically the thickness of the skin and scaling. The effectiveness of the extract or the retinoic acid was indicated by the level of smoothness of the skin. The improved histological characteristics of the skin was accounted to the extract's efficacy. According to investigations elsewhere (Bos, 1997), the extracts thus showed good antipsoriatic efficacy by regulating metabolism, endogenous homeostasis, and sensory input to the skin in addition to actively participating in immunological regulatory and inflammatory responses. This suggests that the antipsoriatic effects of *D. hamiltonii* roots extract are the result of a synergistic interaction between its phytochemicals. Our results corelates with other researchers as well (Asogwa *et al*., 2020).

An established and reliable *in vivo* experimental method for examining the efficacy of anti-inflammatory medications, the model of paw oedema caused by carrageenan was used. According to this paradigm, oedema develops during the course of two separate phases (Vinegar and Waheed, 1987). Previous studies have shown that prostaglandins (Habib *et al*., 2013) are released at the late period, and during the early phase, inflammatory mediators like bradykinins, serotonin, and histamine are produced. Because of these processes, there is an increase in vascular permeability, which makes it easier for neutrophils to infiltrate and for plasma fluid to build up in the interstitial space, which can result in oedema. Our current investigation illustrated the noteworthy anti-inflammatory effects of EEDHR that may be attributed to decreased infiltration of leukocytes, the study also corelates with other study as well (Asogwa *et al*., 2020). The phytochemicals included in EEDHR are responsible for the plant's anti-inflammatory properties, especially the flavonoids that scavenges the free radicals and regulates the cellular activities of inflammation related cells, like mast cells, macrophages, lymphocytes and neutrophils. Other work has also shown the ability of flavonoids in scavenging the free radicals and preventing from cellular activities of the inflammed cells (Vinegar *et al*., 1987). Previous study has also exhibited notable anti-inflammatory property of EEDHR (Ashalatha *et al*., 2010; Ghosh *et al*., 2011).

Sanjay *et al*. (2022) have demonstrated the antioxidant, anti-inflammatory and immunomodulatory potential of Ashwagandha FBC extract due to presence of withanoside-4 and withanolide-A. Haematological status is considered as a key index for diagnosis. Change in the haematological parameters may be positive or negative depending upon the drugs or phytochemicals administered orally (Ajagbonna *et al*., 1999). In the present study, *D. hamiltonii* roots extract decreased the TWBC count but not below the normal values. We also observed an increase in levels of PCV, Hb and RBC. Some argue (Brosche *et al.* 1990) that the alteration of blood parameters could have been due to toxic chemical exposure but other researchers have proved that some phytochemical have the ability to encourage or prevent the production of WBC or RBC. The phytochemical (St John's worth) that were lipophilic exhibited immunosuppressive activity regarding humoral and cellular immunity response (Pulok *et al.,* 2014). TNF-α and IL-2, two proinflammatory cytokines, are overexpressed in several inflammatory processes (Wong *et al*., 1996; Dinarello *et al*., 2002). Sundar *et al*. (2016) also noted that psoriasis management is through immunosuppressant, clinically. So, any attempt to decrease these cytokine expressions can be vital in the treatment of psoriasis. Phytochemicals isolated from the roots of *D. hamiltonii* showed anti-inflammatory properties by down regulating mRNA specific to IL-2 and TNF-α (Mayser *et al*., 2008). The decrease in TWBC by the extract indicates that there is the possibility of immunosuppressive activity through the suppression of proinflammatory cytokines production, that has been proved elsewhere (Ashalatha *et al*., 2010). Further in our study, the rats assigned to receive the EEDHR or retinoic acid showed a progressive decline in the psoriasis severity index and that is similar to other study conducted by Mukul Sharma and Mukesh Chandra Sharma (2023).

Histopathological results showing normal keratinocytes, epithelialization, and substantial collagen fiber synthesis suggest that the extract stimulates healing responses. This implies that *D. hamiltonii* root extract might aid in tissue regeneration and repair, which is advantageous when treating inflammatory skin diseases like psoriasis. Naturally occurring polyphenols (flavonoids) are well established for their strong antioxidant property. Several isolated phytochemicals function as anti-inflammatory and anti-proliferative medicines by modulating a number of signaling pathways. These features may prove beneficial in the management of oxidative stress-related multi-cause disorders, such as psoriasis. In a previous study, Perry's mouse tail model demonstrated a noteworthy decrease in epidermal thickness in response to the flavonoid, according to Vijayalakshmi *et al*. (2012). The presence of different phytoconstituents, particularly flavonoids in the extract, may be responsible for the reported antipsoriatic effect, as it may suppress keratinocyte growth.

5. Conclusion

Ethanolic extract of *D. hamiltonii* roots showed promising and safe pharmacological properties along with anti-inflammatory, antipsoriatic, and wound-healing effects. Biologically active phytochemicals such as alkaloids, flavonoids, fatty acids, and other naturally occurring polyphenols can modulate certain bodily cells such as mast cells, lymphocytes, macrophages, and neutrophils that are involved in the inflammatory processes. These phytochemicals have been testified for the management of oxidative stress-related multi-cause disorders, such as psoriasis. To establish effectiveness and safety of this extract in humans, detail research is required through isolation of phytochemicals that are effective in alleviating the symptoms of psoriasis.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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