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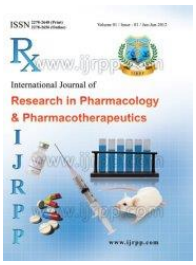

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Antipsoriatic activity of ethanolic extract of *morinda reticulata* in a novel in vivo screening model

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ABSTRACT

The goal of this work was to use an unique in vivo screening strategy to examine the antipsoriatic effect of ethanolic extract of Morinda reticulata (EEMR). For psoriasis induction, 0.1 ml of prepared complete Freund's adjuvant (CFA) & formaldehyde mixture (1:10) was topically administered to the dorsum surface of Swiss albino mice's skin for 7 days. The phenotypic (redness, erythema, and scales) & histological aspects of psoriasis severity index (PSI) were assessed (epidermal thickness). After inducing psoriasis, the therapeutic impact of 0.05 percent and 0.1 percent (w/w) EEMR ointments was examined. The antipsoriatic activity of EEMR ointments was assessed using the PSI and histological investigation after they were applied once daily for three weeks. In animals treated with 0.05 percent and 0.1 percent (w/w) ointments of EEMR, we observed phenotypic and histological features & found a progressive reduction (P 0.05) in the severity of psoriatic lesions (redness, erythema, & scales) from day 7 to day 21 as well as decreased epidermal thickness. The findings revealed that 0.1 percent (w/w) EEMR ointments have dose-dependent therapeutic effects in CFA & formaldehyde-induced psoriasis. The current study discovered that *M.reticulata* has substantial antipsoriatic activity and can be used to treat psoriasis.

Keywords: Flavonoids, Formaldehyde, Inflammation, Psoriasis, *Morinda reticulate.*

INTRODUCTION

Psoriasis is indeed an immune-mediated, skin conditions illness characterised by red, thicker plaques with silvery-white scaly patches overlaying them, which are mostly found on extensor areas but can also affect the palms and scalp. [1] Psoriasis is a chronic disease that has unpredictable remissions and relapses, impairing the sufferers' psychosocial lives. [2] Despite the fact that genetic, immunological, & environmental variables appear to be involved, the specific aetiology is unknown, and psoriasis remains a severe worldwide problem nowadays. [3] There are several therapeutic options available, however the cost, availability, and

adverse effects of long-term usage of synthetic medications for psoriasis are still a concern. [4] Herbal formulations are generally less expensive and are believed to reduce the risk of adverse effects, making them a potential psoriasis therapy option. [5]

Morinda reticulate belongs to the family of Rubiaceae, It is large woody climbing shrubs. Leaves 6-12 x 2-4.5 cm, oblanceolate to linear-lanceolate, attenuate at base, caudate acuminate at apex, waxy shining above, lateral nerves 10-12 pairs; petioles to 6 mm long; stipules acute, connate. white in terminal umbellate heads; peduncle 1-2 cm. Calyx truncate, limb forming a ring. Corolla rotate; tube c. 1.5 mm long, very hairy within; lobes 4, oblong, recurred. Stamens 4, included. Stigma 2-fid. Syncarpium irregularly lobed, 0.5-1 cm diam., with prominent scars of calyx ring, orange; pyrenes many, bony, pyriform, triquetrous in viscous pulp. Habit: Climber, Flowering & Fruiting: March-September, It was found in Choodal, Kallar, Kulathupuzha, Kottayali, on way to Nilamel, Boneccord, Kottur R.F., Merchiston, Thenmalai, Bonaccord, Karamanayar region (6,7). In traditional Japanese, Korean and Chinese medicine, Morinda citrifolia is considered to be an herb with biological properties, although there is no confirmed evidence of clinical efficacy. However, till date, there are no validated scientific reports for its antipsoriatic activity. Therefore, in light of ethnopharmacological facts of the plant, the aim of the present study was to investigate the antipsoriatic potential of ethanolic extract of *M.reticulata* (EEMR) in complete Freund's adjuvant (CFA) and formaldehyde-induced psoriasis.

Plant Material

The plant Morinda reticulatae Gamble hook was found in Madurai, Trichy District's western ghat. During the month of November 2021, plant collecting were completed. Dr.P.Jayaraman, professor at PARC in West Thambaram, Chennai, certified the plant. The plant's voucher specimen was kept at the college for future reference.

Extraction

M reticulata areal pieces were shade dried for two weeks, then pulverised to a coarse powder and sieved no. 20 to ensure homogeneity. The coarsely dried powder was first defatted with petroleum ether (60-80°C) for 72 hours to remove fatty materials, and then extracted with ethanol (95%) using the Soxhlet apparatus for 36 hours. The extract was collected, filtered through Whatman filter paper, concentrated in vacuum under reduced pressure using a rotary flash evaporator, and the dried extract was stored in an airtight container at 4°C for further study. The extract's percentage yield was calculated.

Preliminary Phytochemical Screening

Standard protocols were used to put EEMR to various phytochemical screening tests in order to identify the phytoconstituents contained in M reticulata. [8]

Determination of Total Polyphenolic and Flavonoid Contents

The total polyphenol content of the EEMR was determined using the Folin-Ciocalteu technique and ultraviolet spectrophotometry. [9] In a test tube, 0.1 ml of the extract solution was combined with 0.5 ml of Folin-Ciocalteu reagent, and 3 ml of distilled water was added. 2 ml of 20 percent sodium carbonate solution was added after 3 minutes of incubation and properly mixed. The resulting mixture was heated to 50°C for 5 minutes before cooling to room temperature. The mixture's absorbance was measured at 650 nm against a reagent blank. All of the measurements were done three times. Using the linear equation derived from the calibration curve of the standard gallic acid graph, the amount of phenolic compounds was represented as mg of gallic acid equivalents (GAEs)/g of dry extract. The correlation coefficient (R2) was 0.9971.

The aluminium chloride (AlCl 3) technique was used to determine the total flavonoid content of the EEMR. [10] A volume of 0.5 ml AlCl 3 ethanol solution (2%) was added to a volume of 0.5 ml sample solution. At a final concentration of 0.1 mg/ml, the extract sample was tested. The absorbance was measured at 420 nm after 1 hour of incubation at room temperature. All of the measurements were done three times. Using the linear equation obtained from the calibration curve of the standard quercetin graph, the total flavonoid content was estimated as mg of quercetin equivalents (QEs)/g of dry extract. The correlation coefficient (R2) was 0.9964.

Chemicals and Reagents

Janssen-Cilag Pharmaceuticals provided Retino-A 0.05 percent cream (Tretinoin Cream, USP) (Trademark of Johnson & Johnson, USA). Sigma-Aldrich, USA, provided CFA, gallic acid, quercetin,

and Folin-Ciocalteu reagent. All of the remaining chemicals, including the solvent, were purchased from HiMedia Pvt. Ltd. in Mumbai, India.

Animals

Healthy Swiss albino mice, aged 4 months and weighing between 25 and 30 g, were used in the experiment. Truba Institute of Pharmacy in Bhopal, Madhya Pradesh, provided the animals. During the study, the animals were acclimatised to conventional laboratory settings in a cross-ventilated animal housing at 25°C 2°C, relative humidity 44-56 percent, and light and dark cycles of 12:12 h. They were fed a regular meal and water ad libitum. According to the Committee for the Purpose of Control and Supervision of Experiments on Animals rules, the study protocol was approved by the Institutional Animal Ethics Committee.

Induction of Psoriasis

Antipsoriatic medicines have been evaluated using the mouse tail model for psoriasis. [11] For the production of psoriasis, we established a modified in vivo screening paradigm using a combination of CFA and formaldehyde. The model is based on the ability of CFA to stimulate the immune system and cause inflammation, as well as the usage of formaldehyde as a phlogistic agent that could enhance CFA's inflammatory effects. A stable combination of CFA and formaldehyde (1:10) was developed [12],[13]. Depilatory cream was used to remove hairs on the dorsum area of each mouse (almost 2 cm 2 cm) (Reckitt Benckiser, Inc., UK). At days 1, 2, and 3, a volume of 0.1 mL of the produced combination was applied topically to the shaved area of all test animals (n = 10)

For seven days, the animals were monitored for psoriatic lesions. The clinical psoriasis area and severity index were used to create an objective scoring system. [14] On a scale of 0 to 4, redness, erythema, and scales were rated separately: 0, none; 1, faint; 2, moderate; 3, marked; and 4, extremely marked. The psoriasis severity index (PSI) was calculated using the cumulative score (the total of redness, erythema, and scaling) (scale 0-12). Animals were sedated with ketamine at the end of the study, and skin samples were obtained and kept in glass vials containing 10% formalin solution for histological investigation. Microtomy was used to create longitudinal sections of mice skin specimens (about 5 m thickness), which were then stained with hematoxylin and eosin (H and E) dye for histological evaluation.

Preparation of Test Formulations

Stearyl alcohol, white petrolatum, and liquid paraffin were used as the oleaginous phase, and sodium lauryl sulphate, ethylparaben, glycerin, and water were used as the aqueous phase to make simple ointments of EEMR, 0.05 percent and 0.1 percent (w/w).

Acute Dermal Toxicity Study

The acute cutaneous toxicity of EEMR-prepared ointments was assessed using guidelines no. 402 of the Organization for Economic Cooperation and Development. [15] The Swiss albino mice were split into two groups, each with six animals. Hairs were removed from 10% of the body surface area on the dorsum part of the body 24 hours before the test. On the shaved area, a starting dose of 2000 mg/kg body weight of prepared 0.05 percent and 0.1 percent (w/w) ointments was administered topically. Redness, erythema, changes in fur, sleep pattern, behaviour pattern, and mortality were all monitored for 14 days in both groups of treated animals.

Antipsoriatic Activity of Test Formulations

The animals were given psoriasis by topically administering a mixture of CFA and formaldehyde, as described in "Psoriasis Induction." To lessen the error in mean PSI between the groups after induction, all of the animals were re-randomized prior to treatment. The diseased animals were separated into four groups, each with six animals (n = six). Group I was left untreated, Group II was given Retino-A cream (0.05 percent), and Groups III and IV were given 0.05 percent and 0.1 percent (w/w) EEMR ointments, respectively. After inducing psoriatic lesions in the animals once a day for three weeks, they were given a treatment. Every week, the severity of psoriatic lesions was scored to see if the symptoms of psoriasis had improved. On a scale of 0 to 4, redness, erythema, and scales were rated separately: 0, none; 1, faint; 2, moderate; 3, marked; and 4, extremely marked. The PSI was calculated using the cumulative score (the sum of redness, erythema, and scaling) (scale 0-12). Animals were sedated with ketamine at the end of the study, and skin samples were taken and kept in glass vials containing a 10% formalin solution. Microtomy was used to generate longitudinal sections of mice skin

specimens (about 5 m thickness) for histological evaluation.

Statistical Analysis

The Tukey-Kramer multiple comparisons test was used to assess all of the experimental outcomes, which were reported as mean standard error of mean. GraphPad Software Inc, San Diego, CA, USA, was used to perform the statistical calculations. In all cases, P 0.05 was considered statistically significant.

RESULTS

Acute Dermal Toxicity Study

The ointments were shown to be harmless in a dermal toxicity evaluation. Throughout the research period, there was no fatality or evidence of hazardous responses at the highest dose levels of 2000 mg/kg body weight.

Preliminary Phytochemical Screening

The EEMR percentage yield was discovered to be 8.62 percent (w/w). Flavonoids, glycosides, alkaloids, tannins, triterpenoids, polyphenols, carbohydrates, and proteins were found in the EEMR's qualitative phytochemical examination.

Total Phenolic and Flavonoid Contents

The total phenolic and flavonoid content in the EEMR was found to be 276.13 ± 1.48 mg of GAEs/g of the dry extract and 31.74 ± 0.24 mg of QEs/g of the dry extract, respectively.

Induction of Psoriasis

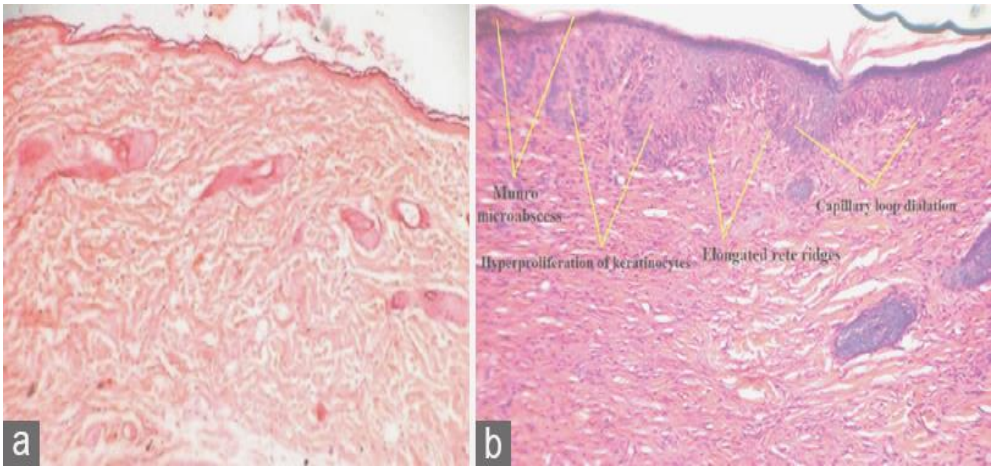
The formation of induced psoriasis on the dorsum part of the mice was induced by topical administration of 0.1 ml of CFA and formaldehyde for 7 days. Several phenotypic changes on exposed areas, such as redness, erythema, and silvery scales, were visibly observed and shown to grow in severity, whilst the cumulative PSI score was significantly (P 0.05) raised on the seventh day after induction, as shown in [Table 1].

Table 1: Examination of redness, erythema, and scales in complete Freund's adjuvant- and formaldehyde-treated mice.

Day	Redness	Erythema	Scales	Cumulative score (PSI)
1	1.02±0.04	-	-	1.02±0.04
2	1.40±0.07	0.42±0.07	-	1.83±0.15
3	1.75±0.07	1.00±0.09	-	2.76±0.17
4	2.17±0.08	1.68±0.05	0.80±0.07	4.67±0.22
5	2.60±0.06	2.21±0.07	1.76±0.07	6.59±0.21
6	2.93±0.03	2.58±0.05	2.33±0.09	7.86±0.19
7	3.24±0.03	2.86±0.05	2.78±0.08	8.90±0.18

The results represent mean±sem. Data were analyzed by one-way ANOVA, Followed by Tukey-Kramer multiple comparisions test, values were considered significant at *P<0.05 and **P<0.01. ANOVA: Analysis of variance, PSI: Psoariasis Severity index, CFA: Complete Freund’s adjuvant, SEM: Standard error if mean.

Histological examination revealed increased epidermal thickness, keratinocyte hyperproliferation, granulocyte infiltration, the presence of Munro's microabscess, capillary loop dilatation, elongation of rete ridges, and the absence of the granular cell layer (parakeratosis) in CFA- and formaldehyde-treated mouse skin compared to normal mouse skin [Figure 1]. a and b are two options. All of these phenotypic and histological characteristics are strikingly similar to human plaque psoriasis, indicating that this is a true psoriasis mouse model.



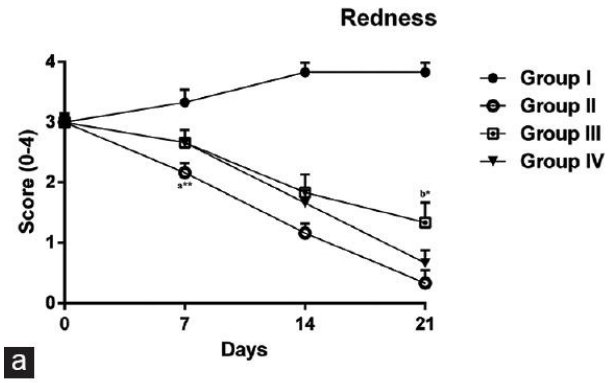
(a) normal mouse skin section and (b) full Freund's adjuvant- and formaldehyde-treated mouse skin section. (Munro's microabscess, keratinocyte hyperproliferation, enlarged rete ridges, and capillary loop dilatation are indicated by the arrows.)

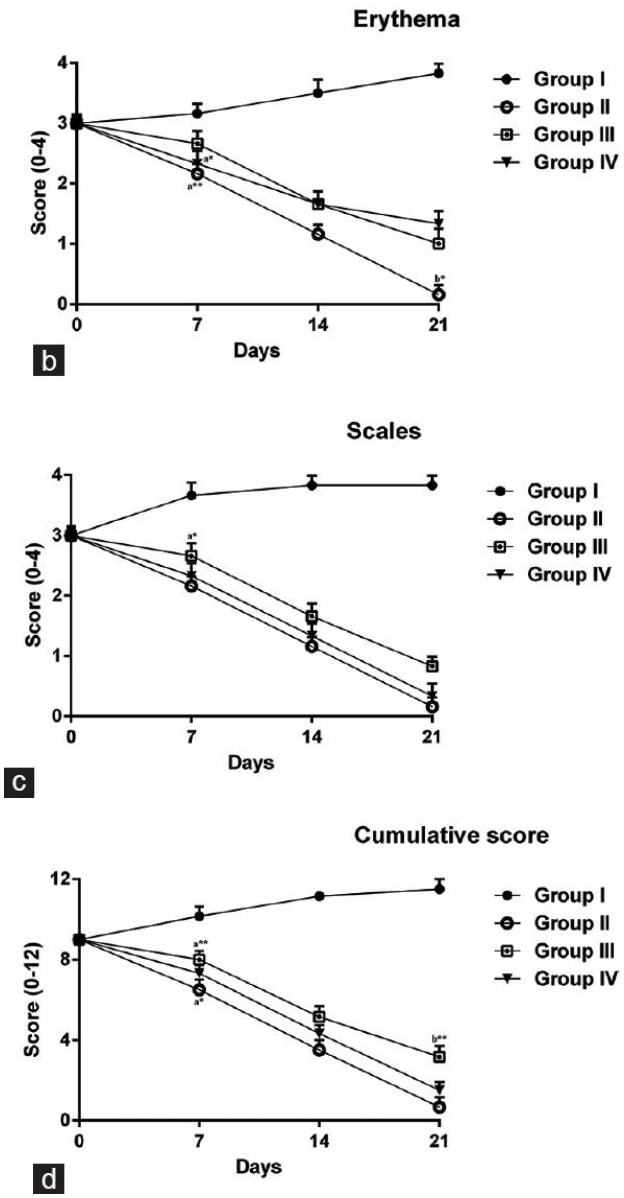
Fig 1: Longitudinal histological sections of mouse skin (H and E, 40)

Effect of Test Formulations on Complete Freund's Adjuvant and Formaldehyde-induced Psoriasis

Following psoriasis induction, 0.05 percent and 0.1 percent (w/w) EEMR ointments were applied once daily for three weeks, and the severity of psoriatic lesions was assessed by visual and histological investigations. [Figure 2] shows the results. Throughout the experimental period, the severity of psoriatic lesions (redness, erythema, and scales) in the untreated group (Group I) was steadily increased in ocular examinations, which was repeatable in all animals [Figure 2]. a-c. On the 21st day, the cumulative score in Group I [Figure 2]d was considerably (P 0.05) higher than in the other groups.

From day 7 to day 21, topical administration of Retino-A cream (0.05 percent) reduced the severity of redness, erythema, and scales in Group II (P 0.05). The therapeutic impact of a typical medicine on psoriatic lesions was demonstrated by the cumulative score in Group II [Figure 2]d. In comparison to Group I [Figure 2]d, administration of 0.05 percent (w/w) ointment resulted in a steady decrease in redness, erythema, and scales [Figure 2]a-c, as well as a substantial reduction (P 0.01) in the cumulative score. In Group IV animals, a 0.1 percent (w/w) ointment was found to have a substantial therapeutic effect (P 0.001) on psoriatic lesions. The therapeutic efficacy of M reticulata on induced psoriasis was demonstrated by the reduction of redness, erythema, scales, and cumulative score in animals [Figure 2]d.



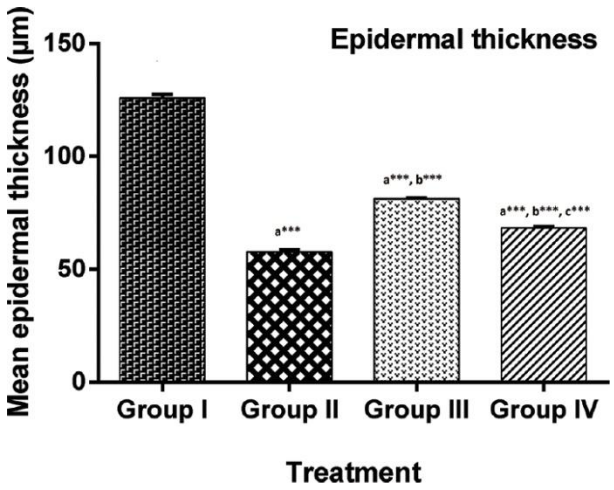


*(a) redness, (b) erythema, (c) scales, and (d) cumulative score were observed. The results are the mean minus the standard error of the mean for six mice in each group. Data were examined using one-way analysis of variance, followed by the Tukey-Kramer multiple comparisons test; significant differences were defined as *P 0.05 and **P 0.01, a-significant difference compared to Group I, and b-significant difference compared to Group II.*

Fig 2: In the extract of Morinda reticulata-treated mice, changes

Histopathology

In comparison to the standard and treated groups, increased epidermal thickness of approximately 2-fold [Figure 3], hyperproliferation of keratinocyte cells, and the presence of other features such as granulocyte infiltration and neutrophil collection beneath the epidermis were indicators of psoriasis in untreated animals. Based on histological investigation and epidermal thickness, EEMR-treated mice demonstrated a great therapeutic effect. In terms of reduced parakeratosis [Figure 3] and granular layer retention, epidermal thickness was considerably reduced (P 0.001), indicating reduced keratinocyte hyperproliferation and keratinization beginning.



*Each bar indicates the mean minus the standard error of the mean for each of the six mice in each group.*P 0.05, **P 0.01, and ***P 0.001, a-significant difference compared to Group I, b-significant difference compared to Group II, c-significant difference compared to Group III; values were substantially different from those of the control group: *P 0.05, **P 0.01, and ***P 0.001.*

Fig 3: Changes in epidermal thickness in mice treated with a Morinda reticulata extract

DISCUSSION

The specific cause of psoriasis is unknown because it has numerous etiologies. [3] In recent years, a great deal of research has gone into elucidating the exact aetiology of psoriasis and developing new therapy options. However, the lack of a direct and efficient in vivo screening paradigm is a key problem in the discovery of novel drugs for the treatment of psoriasis. Existing animal models, such as the mouse tail model and the xenograft model, necessitate a high level of competency and technical expertise, and they are less convenient in terms of cost and availability. [11] The capacity of CFA to generate the precise immunological response created via influx of the various cells of the immune system was the explanation behind the choice of CFA and formaldehyde to induce psoriasis. [16],[17] Formaldehyde was chosen as a phlogistic agent that may provoke an inflammatory response by increasing the release of several chemical mediators, hence amplifying the action of CFA. [18].

Intraepidermal penetration of activated polymorphonuclear leukocytes produces uncontrolled formation of reactive oxygen species, causing peroxidative damage to skin membranes and contributing to the worsening of lesions in early and active psoriatic lesions. Reactive oxygen species can also activate phospholipase A2, increasing the release of arachidonic acid mediators. The cyclooxygenase system produces prostaglandin E2, which dilates capillaries in the dermis, increases leukocyte infiltration, and stimulates keratinocyte cell development, all of which contribute to psoriasis. [19]

Topical administration of CFA and formaldehyde to the skin of mice induced various pro-inflammatory reactions (redness, erythema, and scales) during psoriasis induction. Inflammatory erythematous papules covered in dry silvery and red scales characterising fully formed psoriatic lesions; this condition resembles a parakeratotic state accompanied with an obvious evidence of plaque psoriasis, indicating a precise immune response caused by the CFA. [16],[17],[20] The severity of psoriatic lesions suggests that formaldehyde has a function in inducing inflammation to enhance the action of CFA. [18] Psoriasis is characterised by epidermal keratinocyte hyperproliferation and abnormal differentiation, lymphocyte infiltration, primarily T-lymphocytes, and specific endothelial vascular changes within the dermal microvasculature, including limited neoangiogenesis, capillary dilation, and high endothelial venules formation. Histological examination of the CFA and formaldehyde-treated mice skin revealed that all of these [21].

Herbal medications have been the subject of a lot of research in recent years to see if they may help with psoriasis. Herbal medicine appears to be a potential alternative treatment for psoriasis at the moment. [22] Previous research has suggested that antioxidants may be useful in the treatment of psoriasis. [23] Antioxidants such as flavonoids, triterpenoids, and

polyphenolic chemicals are well known for their anti-inflammatory, antiproliferative, immunomodulatory, and free radical scavenging properties. [24],[25] These properties of polyphenolic phytoconstituents could be useful in the treatment of disorders having numerous causes, such as psoriasis. The presence of a large amount of flavonoids and polyphenols in M reticulata was discovered through phytochemical screening and standardisation of the EEMR. Despite the fact that psoriasis is a repeated chronic inflammatory skin illness, EEMR showed a protective impact in battling psoriasis through many mechanisms, which is more important than other medications that work through a single mechanism.

CONCLUSION

When compared to normal, the developed in vivo screening model was defined by phenotypic and histological investigation. Along with the heightened PSI, CFA and formaldehyde-treated mice developed increased redness, erythema, and scales. The similarity of typical psoriatic lesions to human plaque psoriasis has also been confirmed by histological findings. Induced psoriasis caused by the topical application of a mixture of CFA and formaldehyde could be used as a legitimate screening model to assess medication antipsoriatic activity. Both EEMR test formulations (0.05 percent and 0.1 percent ointment) reduced psoriasis symptoms as well as mean PSI. The presence of a large amount of bioactive phytoconstituents (flavonoids and polyphenols) in the ethanolic extract was found to be responsible for the antipsoriatic action of *M.reticulata*. These findings show that *M.reticulata* could be used to treat psoriasis, confirming its historic use in skin problems. More research is needed to learn more about the separated phytoconstituents.

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