Assessment of Anti-atopic Dermatitis Activity of Oroxylum indicum Extract Incorporated Cream

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Authors’ contributions

This work was carried out in collaboration between both authors. Author DHTT conducted literature search, performed all experiments and wrote the manuscript. Author HLS conceptualized, supervised the study and revised the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: The search for plant-based treatments against atopic dermatitis (AD), a relapsing dermatological condition with high prevalence in tropical regions, has always been attracting special attention. Among many folk remedies for AD, Oroxylum indicum Linn (Bignoniaceae) is a commonly used medicinal plant whose effectiveness has not yet been scientifically reported. This study thus aimed to investigate the anti-AD activity of ethyl acetate extract from the bark of Oroxylum indicum.

Methodology: Five cream formulations containing Oroxylum indicum ethyl acetate bark extract in different concentrations (0%, 1.25%, 2.5%, 3.75%, and 5%) were topically applied onto the dorsal skin of 2,4-dinitrochlorobenzene-sensitized BALB/C mice once a day during 6 weeks. Phosphate-Buffered Saline (PBS) and Protopic (Tacrolimus 0.1%) were used as negative and positive control, respectively. All mice were subjected to the assessment of AD-like symptoms including the development of eczematous skin lesions, intensity of pruritus and histological alterations.

Results: The plant extract at 5% was significantly effective in suppressing the dermatitis scores by 23.26% (n=6, p<0.001) and scratching frequency by 34.86% (n=6, p<0.001) compared to the

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negative control while markedly reducing the signs of parakeratosis, hyperplasia, spongiosis, acanthosis, as well as the epidermal and dermal thickness in immunized mice. **Conclusion:** These results confirmed the inhibitory effect of *Oroxylum indicum* on the advance of AD at 5% when incorporated into a cream formulation and revealed the plant’s therapeutic potential in the approach to disease treatment. However, more studies on the immunosuppressive mechanism of the extract and the interplaying roles of main phytocomponents should ultimately support the utilization of this valuable medicinal plant.

**Keywords:** 2,4-dinitrochlorobenzene; *Oroxylum indicum* bark extract; dermatitis score; scratching behavior; histological alterations; medicinal plant.

### 1. INTRODUCTION

#### 1.1 Atopic Dermatitis

Atopic dermatitis or atopic eczema is a chronic skin inflammatory disease that occurs commonly in either infancy or adulthood with the hallmark of intense pruritus [1]. Various AD symptoms including erythematous eczematous skin lesions, lichenification, xerosis, cutaneous hyperactivity and hyper keratosis in both intrinsic and extrinsic AD types manifest through acute, subacute and chronic AD cause serious inconvenience for patients in daily life [2]. Predisposing factors interplaying in the disease development include genetics, immune-mediated responses and provocations from environmental factors such as microorganism, irritants, allergens, food and climate [3]. AD prevalence was reported to be comparatively high among dermatological conditions in many communities in Africa, Asia-Pacific and Indian Subcontinent and even rising in certain urbanizing countries belonged to these regions [4-6]. To make such endemic increase more problematic, the symptoms of AD are often aggravated by tropical environment due to its distinctively large amount of heat and high risk of bacterial or fungal infestation [7]. The number of AD incidence in some tropic regions featured with cold and arid atmosphere such as mountainous areas in the north of Indian is also remarkably high [8]. Indeed, the search for a novel and effective anti-AD agent deserves special attention considering its convoluted, frustrating nature and wide span of endemic areas.

Immunological responses, specifically excessive activation of T cell in inflammatory regulation that leads to hypersensitive reactions, are the central of AD pathogenesis. The treatments of AD, thus, aim to ameliorate the immune dysregulation caused by imbalance ratio of Th1/Th2, reduce of pruritus intensity and recover functionality of the skin barrier, which is damaged by prolonged inflammation [9]. However, existing AD treatments are bound to certain concerns that could negatively affect patients’ adherence to the treatment: The use of corticosteroids as standard management associates with phobia of local side effects like skin atrophy, striae, steroid acne or increased hair growth and systemic side effects such as dwarfism, cataract or Cushing’s syndrome [10-13]. Topical calcineurin inhibitors might cause immunosuppression or skin irritating effect while other systemic therapies can lead to adverse events like headache, nausea, renal impairment or hypertension [14]. Accordingly, a serious demand for discovery and development of complementary approaches to AD has been imposed, promoting the practice of traditional methods and the utilization of naturally derived materials to a great extent [15].

#### 1.2 Oroxylum indicum L.

*Oroxylum indicum* Linn (Bignoniacaeae) (*O. indicum*) is a plant with reported use as a traditional AD treatment. The plant, known as midnight horror or Indian trumpet flower plant distributed throughout different areas of Southeast Asia. Each part of this plant is used as folk treatment. *O. indicum* root bark is an astringent and bitter tonic used for treating bronchitis, diarrhea or fever [16]. Its seeds are used as remedies for stomach disorders and wounds, when its barks can alleviate diarrhea, dysentery, ulcer and eczema [17].

A large number of pharmacological reports on *O. indicum* have been conducted. Its root barks possess anti-inflammatory, analgesic, antimicrobial, antiulcer, immunomodulatory, antioxidant, cytotoxic, and apoptotic [18-20]. *O. indicum* leaves possess hepatoprotective effect, nephroprotective and anti-diabetic effects [21]. Such a wide range of pharmacological activities are attributable to *O. indicum* phytochemical composition which contains a high amount of tannins, glycosides, phenolics, phenolic acids and flavonoids [22]. Four flavonoids were isolated from the leaves extract, including chrysin, hispidulin, baicalein and oroxylin A [23].
Also from the leaves, quercetin-3-o-α-L-arabinopyranoside, 1-(2-hydroxyethyl)cyclohexane-1, 4-diol, and apigenin were isolated [24].

Although the use of *O. indicum* to treat AD has been widely passed through words of mouth for generations in many countries where medicinal plants are highly appreciated like India, China and Vietnam, no scientific evidence is in existence to verify the plant's potential as an anti-atopic dermatitis agent. As mentioned above, *O. indicum* is a rich source of phenolics and flavonoids which contribute to its effectiveness in treating inflammatory diseases. This suggests that a research on the effectiveness of *O. indicum* on alleviating AD, a common skin disease whose pathogenesis is closely related to inflammatory responses, should be conducted.

### 2. MATERIALS AND METHODS

#### 2.1 Animals

Female BALB/C mice were procured from Pasteur Institute (Ho Chi Minh, Vietnam) and allowed to acclimate in experimental conditions for 1 week. All animals were maintained in accordance with the Animal Experimental Hand Book at Cellular Reprogramming Laboratory, International University, Vietnam National University of Ho Chi Minh City (http://crl.bio.hcmiu.edu.vn/about-us/facilities/) and additionally with the Guide for the Care and Use of Laboratory Animals (8th edition) [25]. Purchased mice were kept in a controlled environment of 12 h of light and dark cycle, while a standard diet and water were provided *ad libitum*.

#### 2.2 Chemicals and Reagents

Methanol, ethyl acetate, acetone, olive oil, and other analytical chemicals were provided by Natural Products Lab (Institute of Chemical Technology, Ho Chi Minh City, Vietnam). 2,4-dinitrochlorobenzene (DNCB) was purchased from Sigma Chemical Co. (Singapore) and dissolved in the mixture of acetone/olive oil (1:4) to make 1% (w/v) and 0.5% (w/v) solutions. Protopic 0.1% (Tacrolimus ointment) was obtained from a drugstore.

#### 2.3 Preparation of Plant Extract

The bark of *O. indicum* collected in Kon Tum (latitude 14.35° N., longitude 108.00° E., Highland Central of Vietnam, December, 2015) was identified by Associate Professor Dr. Tran Van Minh of the Institute of Tropical Biology, Vietnam and deposited in the herbarium of Applied Biochemistry Laboratory, Department of Applied Biochemistry, School of Biotechnology, International University, Vietnam National University – Ho Chi Minh City, Vietnam with voucher No. HB-BIO-06-08-8. The plant materials were washed with water before being dried in oven at 60°C. Plant materials were then ground into fine powder (2 kg) and macerated with 10 L of methanol for 1 week. After the filtration with No. 2 Whatman filter paper, crude extract was concentrated by rotary evaporation and subjected to the extraction with 10 L of ethyl acetate. The yield of dry matter from the concentrated extract was weighted and stored in refrigerator at 4°C for later use.

#### 2.4 Cream Preparation

Carbomer 940 as a suspension agent was dispersed in distilled water to form a smooth gel named part A. The prepared plant extract was weighted and dissolved completely in a small volume of distilled water in order to prepare cream samples with 0%, 1.25%, 2.5%, 3.75% and 5.0% extract (w/w). Preservatives including sorbic acid and potassium sorbate were then added into the dissolved extract, creating part B. Preparation of the cream's water phase continued as part A and B were combined together with continuous agitation and finished by the addition of propylene glycol. Oxyldodecanol was dispersed in the water phase before distilled water was added to make up 100 g of mixture. Homogenization of this combination was carried out by a homogenizer until a smooth, clump-free cream with good integrity was obtained. pH Values of all obtained formulations were adjusted to 4.5-5.0 (Wiechers et al. 2008) using 0.2% citric acid and 0.1% triethanolamine. No heat was applied during the whole process of preparation. The formulations with different percentages of plant extract were stored in tightly-closed plastic jars and kept in cool place.

#### 2.5 Evaluation of Cream Formulations

##### 2.5.1 Physical observation

Physical characteristics including color, smell and feeling of application of prepared creams were noted.
2.5.2 Homogeneity

All creams were subjected to visual and manual inspections, as the presence of any clump or disintegration was considered signs of heterogeneity.

2.5.3 Determination of pH

pH values were measured using digital pH meter following the dissolution of 0.5 g of cream sample in 50.0 mL of distilled water [26]. The measurement of each cream was triplicated.

2.5.4 Viscosity

The measurement of prepared creams was carried out using Brookfield viscometer Model RV-E (Institute of Applied Material Science, HCM City) with spindle type CP40 at rotation rate of 0.10 rpm at 33.5°C.

2.5.5 Stability study

The stability of cream formulations was evaluated based on the changes in appearance, smell, color and after-feel in a 2-month interval.

2.5.6 Skin irritation

Ten human volunteers in the test for irritation received 1.0 g of each prepared cream for topical application on an area of 1 cm². The participants were asked to record and report any occurrence of swelling, redness or itchiness after 24 h. [21].

Table 1. Formulation of the cream base

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Amount (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active ingredients</td>
<td>≤ 10.0</td>
</tr>
<tr>
<td>Propylene glycol</td>
<td>30.0</td>
</tr>
<tr>
<td>Sorbic acid</td>
<td>0.1</td>
</tr>
<tr>
<td>Potassium sorbate</td>
<td>2.7</td>
</tr>
<tr>
<td>Carborner 940</td>
<td>3.0</td>
</tr>
<tr>
<td>DI water</td>
<td>Qs. 100 (g)</td>
</tr>
<tr>
<td>Octyldodecanol</td>
<td>6.0</td>
</tr>
</tbody>
</table>

2.6 Induction of AD and Topical Treatment on Mice Model

Induction of atopic dermatitis was carried out following a previously used protocol with slight modifications [27]. Briefly, dorsal skin of mice were shaved using depilatory cream before being sensitized with 100 μL of 1% DNCB on day 1, 3, 5 in first week. Animals whose dermatitis scores were not statistically different with similar clinical manifestations were then chosen and randomized in to 7 groups of 6. For the next 6 weeks, mice skin was challenged with 100 μL of 0.5% DNCB thrice a week, simultaneously with daily topical application of treatments. Group I received 100 μL of PBS as negative control. Group II to VI received 100 μL of 0%, 1.25%, 2.5%, 3.75% and 5% cream, respectively. Group VII received treatment with Protopic as positive control. All mice were euthanized by inhalation of diethyl ether in week 6 for the purpose of skin histological examination.

2.7 Scratching Behavior Observation

Scratching behavior of mice in terms of the number of scratching bouts was recorded following previously described method [28]. For each mouse, only back scratching with hind paws was counted in 20 minutes by visual observation once a week before the exposure to DNCB.

2.8 Evaluation of Skin Severity

During the challenge and topical treatment of plant extract-loaded cream, the development of AD was assessed based on 4 clinical parameters including erythema/hemorrhage, edema, excoriation/erosion, and scaling/dryness that were marked on the scale of 0-3, as 0 (none), 1 (mild), 2 (moderate), and 3 (severe) following Eczema Area and Severity Index (EASI)–lesion severity atlas [29]. Dermatitis score therefore ranged from 0 to 12 as sum of severity points of these parameters and was evaluated blindly by two individuals once every week.

2.9 Histological Analysis

Skin biopsies were collected on the last day of week 6 and immediately fixed in 10% neutral buffered formalin for 12 hours. The samples were then dehydrated by alcohol in graded concentration (50-100%), cleared in xylene and embedded in paraffin wax before being sectioned at a thickness of 4 μm for the purpose of H&E staining. Signs of acanthosis, spongiosis and parakeratosis were recorded. The thickness of epidermal and dermal layer was measured to assess the degrees of hyperplasia and hyperkeratosis. Procedures were performed under supervision and guidance of a doctor (Histopathology Department, University of Medicine, Ho Chi Minh City).
2.10 Statistical Analysis

All the results were expressed in terms of Mean ± Standard deviation (SD). t-test, one-way and two-way analysis of variance (ANOVA) for comparisons between groups were conducted. Significance level was set to be less than 0.05. GraphPad Prism 6 was used for data analysis.

3. RESULTS

3.1 Preparation of Plant Extract

The total extract from maceration of 2 kg of finely ground powder of materials weighed 165 g. Partition of this crude extract with ethyl acetate yielded 19 g extract that was incorporated into prepared cream base in different concentrations of 1.25%, 2.5%, 3.75% and 5%.

3.2 Evaluation of Cream Formulations

The cream base was an opaque white, shining and easy to spread product with firm smoothness. Other extract-loaded formulations were green and also had listed characteristics along with a pleasant herbal smell. All creams gave a cooling sensation and good adherence on skin, while they could be easily removed by tap water. The homogeneity, pH values, viscosity, and stability of cream formulations were summarized in Table 2, when result of irritation study was presented in Table 3. After 2 months of storage, the homogeneity of the formulations was maintained, despite slight increases in viscosity and decreases in pH.

3.3 Evaluation of Skin Severity

In response to repetitive exposure to DNBC, acute AD-like symptoms such as the formation of pustules with consequent swelling, redness and erosion gradually aggravated skin condition, before lichenification, a chronic symptom, exhibited. These features were well-illustrated in mice from negative control group and appeared to be less severe in others. Skin conditions of extract-treated mice were distinguishable from the negative control, as crust formation, skin dryness and eczematous areas were visibly lessened. For positive control mice which received topical application of Protopic 0.1%, all acute and chronic AD-like symptoms was only mildly severe (Fig. 1A).

Table 2. Physical characteristics of cream formulations in the duration of 2 months

<table>
<thead>
<tr>
<th>Cream base (0%)</th>
<th>Color</th>
<th>Appearance</th>
<th>Homogeneity</th>
<th>pH</th>
<th>Viscosity (cP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>White</td>
<td>Smooth, opaque</td>
<td>Good</td>
<td>4.70±0.06</td>
<td>15859.50±34.78</td>
</tr>
<tr>
<td>After 2 months</td>
<td>White</td>
<td>Smooth, opaque</td>
<td>Good</td>
<td>4.41±0.04</td>
<td>23098.09±72.43</td>
</tr>
<tr>
<td>1.25% cream</td>
<td>Very light green</td>
<td>Smooth, opaque</td>
<td>Good</td>
<td>4.66±0.03</td>
<td>29321.00±102.05</td>
</tr>
<tr>
<td>After 2 months</td>
<td>Very light green</td>
<td>Smooth, opaque</td>
<td>Good</td>
<td>4.51±0.04</td>
<td>31981.23±76.88</td>
</tr>
<tr>
<td>2.5% cream</td>
<td>Light green</td>
<td>Smooth, opaque</td>
<td>Good</td>
<td>4.67±0.05</td>
<td>36678.50±110.23</td>
</tr>
<tr>
<td>After 2 months</td>
<td>Light green</td>
<td>Smooth, opaque</td>
<td>Good</td>
<td>4.58±0.06</td>
<td>42810.91±89.11</td>
</tr>
<tr>
<td>3.75% cream</td>
<td>Green</td>
<td>Smooth, opaque</td>
<td>Good</td>
<td>4.70±0.10</td>
<td>41583.50±78.09</td>
</tr>
<tr>
<td>After 2 months</td>
<td>Green</td>
<td>Smooth, opaque</td>
<td>Good</td>
<td>4.54±0.06</td>
<td>46019.50±92.13</td>
</tr>
<tr>
<td>5% cream</td>
<td>Dark green</td>
<td>Smooth, opaque</td>
<td>Good</td>
<td>4.64±0.07</td>
<td>46216.00±30.41</td>
</tr>
<tr>
<td>After 2 months</td>
<td>Dark green</td>
<td>Smooth, opaque</td>
<td>Good</td>
<td>4.53±0.02</td>
<td>51281.12±92.01</td>
</tr>
</tbody>
</table>

Table 3. Skin irritation testing of cream formulations

<table>
<thead>
<tr>
<th>Cream base (0%)</th>
<th>1.25%</th>
<th>2.5%</th>
<th>3.75%</th>
<th>5%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Edema</td>
<td>No case</td>
<td>No case</td>
<td>No case</td>
<td>No case</td>
</tr>
<tr>
<td>Erythema</td>
<td>No case</td>
<td>No case</td>
<td>No case</td>
<td>No case</td>
</tr>
<tr>
<td>Pruritus</td>
<td>No case</td>
<td>No case</td>
<td>No case</td>
<td>No case</td>
</tr>
</tbody>
</table>
During 6 weeks of investigation, the development of AD was assessed by summing up the score given individually on 4 parameters including erythema, edema, excoriation and lichenification in the scale from 0 to 3 (0 (none), 1 (mild), 2 (moderate) and 3 (severe)). It was not until the last week of treatment that the symptoms in group VI mice were considered significantly different from negative control (23.26%), denoting the superior therapeutic effect of formulation with 5% O. indicum extract compared to those with 1.25%, 2.5% and 3.75% extract. Protopic notably suppressed AD-like symptoms from the 3rd weeks, as it was observable that the severity in group VII was milder than that of other investigated groups (Fig. 1B).

3.4 Scratching Behavior

The number of scratching bouts by hind legs in 20 minutes of each mouse was recorded weekly before DNCB challenge (Fig. 1C). Pruritus, as exhibited through scratching behavior, intensified in correspondence with AD severity in all experimental groups. At the beginning of the treatment period, mice whose dermatitis scores were not significantly different to each other were chosen and randomized into 7 groups. The initial scores of the groups ranged from 1.5 to 2.33 and did not exhibit distinguishable clinical manifestations. It was not until week 6 that scratching frequency in group IV and VI was significantly lower than that of negative control at p <0.05, both by 34.86%. As positive control, Protopic suppressed scratching behavior from week 5 by 46.34% and 51.38% in week 6. However, it was noted that 2 mice in group IV had suffered from hind leg disability and thus, were only able to relieve the itch by biting or rubbing with teeth and front paws. This could be responsible for the group’s significantly low scratching frequency and made it insufficient to conclude about the therapeutic effect of the extract at 2.5%. For all formulations loaded with extract at other concentrations, no significant results as compared to negative control were found. The cream base singly was not effective in suppressing itchiness as high frequency was noted during the whole experiment.

3.5 Histological Analysis

Mice dorsal skin was subjected to staining with H&E dye for the purpose of histological examination. Pustules in the stratum corneum, parakeratosis, hyperplasia, spongiosis and acanthosis with elongation of rete ridges of moderate severity were especially visible in mice treated with PBS, cream base and 1.25% formulations. Such alterations resulted in epidermal and dermal thickening, as well as the appearance of abnormal-shaped hair follicles and sebaceous glands in these skin layers. Exhibited features on the dorsal skin including weepiness, crusting, scaling, thickening and abrasion reflect the intensity of AD-like symptoms on DNCB-sensitized mice. However, those histopathological findings were found to be subdued partly in extract-receiving mice and significantly in positive control mice (Fig. 2A).

In fact, the thickness of epidermis layer in mice treated with plant extract at the concentration of 1.25%, 2.5%, 3.75% and 5% and positive control mice was significantly lower than that of negative control mice (Fig. 2B). Regardless, only the dermal thickness of mice received 5% extract-contained cream and Protopic was markedly reduced as compared to PBS-receiving mice (Fig. 2C). Accordingly, the formulation loaded with the highest concentration of extract showed better efficacy than that of the others in decreasing the epidermal and dermal thickness of DNCB-induced AD mice.

4. DISCUSSION

AD is a complicated relapsing dermatological condition with high prevalence of 15–30% and 2–10% in children and adults, respectively. Manifested through intense pruritus, appearance of dry, eczematous skin lesions, along with inflammatory responses such as irritation, edema and erythema, this disorder stress an enormous burden and frustration on patients in every life, as being recalcitrant in its nature [30]. Responsible for AD development are extrinsic factors like food, climate, exposure to chemicals and allergens or intrinsic factors like inherited abnormality in filaggrin gene expression, hormonal changes, or even psychological stress. These risk factors trigger a chain of inflammatory reactions that exterminates intruding pathogens, yet consequently perturbs immunological balance and exacerbates skin condition if repeated and prolonged. As a result, therapies with immunomodulatory effects, especially those that focus on mediating inflammatory responses, are required for the treatment of AD [14].

O. indicum is a medicinal plant that has been traditionally used as an AD remedy not only in Vietnam but also in other countries where complementary and alternative medicines are
practiced [21,31]. Nonetheless, the therapeutic effect of *O. indicum* against AD has not been scientifically investigated. This plant in fact possesses anti-inflammatory and anti-ulcer activities and might be able to interfere in immunologic responses of eczema pathogenesis [20,32]. This investigation on *O. indicum*’s pharmacological activities, thus, aims to directly clarify the plant’s anti-AD effect and contribute to the utilization of herbal medicines as skin diseases treatment, especially when current standard managements such as corticosteroids, immunosuppressive agents or phototherapy are bound to certain concerns for cost and safety [2]. In this study, *O. indicum* ethyl acetate extract was loaded into a cream carrier at concentrations of 1.25%, 2.5%, 3.75% and 5% and topically applied on BALB/C mice with DNCB-induced AD.

![Image](A) ![Image](B) ![Image](C)

**Fig. 1.** Effects of *O. indicum*-contained cream formulations on DNCB-sensitized mice. (A) Clinical manifestations observed in mice in week 6; (B) Dermatitis severity of experimental groups during treatment period. **Dermatitis score based on signs of erythema, edema, excoriation and lichenification was evaluated once a week.** (C) Scratching behavior during treatment period

*The number of scratching bouts by hind legs was recorded for 20 minutes weekly before sensitization of DNCB. Each point represents the mean (n=6); *p<0.05, **p<0.01 and ***p<0.001 compared to negative control*
Fig. 2. Effects of *O. indicum*-contained cream formulations on mice skin histology. (A) Histological findings by H&E staining. (Magnification: 10x); *Epidermis, dermis and hypodermis layer* were denoted by E, D and H, respectively; *Signs of spongiosis* (→), *parakeratosis* (→→), *pustule* (*) and *elongation of rete ridges* (♦) were presented. (B) Epidermal and (C) dermal thickness of mice dorsal skin.

Each bar represents the Mean±SD (n=6); *p<0.05, **p<0.01 and ***p<0.001 compared to negative control.*

As the carrier of plant extract, the cream base was formulated so that ideal homogeneity, spreadability and applicability were achieved following proposed methods of assessment [26]. All prepared formulations had pH values ranging from 4.5 to 5.0, an acceptable pH span for topical products since it is close to that of human skin [33], and were safe to use as no adverse events were reported during skin irritation test. Overall, the viscosity of these formulations slightly increased within two months, but the formulation’s homogeneity maintained to be satisfying as there was no separation between the oil phase and the water phase. The
formulations were also clump-free and easy to spread, leaving a thin, smooth layer when being applied on skin, after the period of quality examination. However, the cream base was not capable of alleviating dermatitis-like symptoms including itchiness (Fig. 1), and epidermal/dermal thickening (Fig. 2), since no significant improvement compared to PBS treatment was made.

The development of AD symptoms begins with inflammation responses like swelling and redness that are accompanied by formation of blisters and eventually proceeds into dry, scaly and lichenified patches [34]. To assess the severity and progress of DNCB-induced AD-like symptoms in this experiment, dermatitis score (0-12) based on 4 parameters including erythema, edema, excoriation and lichenification was used. These signs were significantly inhibited in animals which received 5% extract-incorporated cream by 23.26% compared to those of negative control, as indicated by their scores in week 6. This illustrates the potential of O. indicum extract at 5% in alleviating AD-like symptoms that are heavily influenced by pro-inflammatory responses.

Pruritus is a hallmark of AD in both acute and chronic phases [14]. To assess the anti-pruritic effect of the plant extract, the number of scratching bouts by hind paws only was recorded weekly in 20 minutes before the challenge of DNCB [28]. Scratching frequency was notably suppressed in mice receiving 2.5% (group IV) and 5% (group VI) extract-contained cream formulations in the 6th week, both by 34.86% (Fig. 1C). Nonetheless, two mice in group IV were found with permanent hind legs injuries due to fighting which forced them to bite the dorsal skin in poor attempts to stop the itch. This could have affected the group’s average scratching frequency and should be disregarded while making a conclusion. Accordingly, the cream containing 5% O. indicum extract was effective in inhibiting pruritus during AD development.

Histopathological findings in negative control animals have well illustrated the skin impairments in AD patients. Specifically, hollow pustules in the stratum corneum were responsible for oozing fluids in reddened, weeping skin at the beginning of an AD remission while hyperplasia, spongiosis and acanthosis were identified as the culprits for skin thickening, erosion and lichenification in chronic phase. As a consequence, defections of skin structures such as parakeratosis, epidermal elongated rete ridges, or hair follicles and sebaceous glands of unusual shapes and sizes in the dermis were visible [1]. These histological changes, however, were less severe in mice topically treated with the plant extract. In fact, the epidermal/dermal thickening of mice from group V and VI was significantly suppressed compared to that of negative control, although only the dermatitis score of group VI was markedly reduced. Epidermal thickness also notably decreased in mice receiving treatments with 1.25% and 2.5% extract cream while dermal thickness did not. Suffice it to say, the formulation of 5% extract showed superiority to those of lower concentrations in suppressing pathological alterations of AD.

Conclusively, the cream containing 5% O. indicum ethyl acetate extract has successfully exerted anti-inflammatory effect on an AD animal model. This was demonstrated as AD-like symptoms including pruritus, clinical manifestations and histopathological features were restrained, although the involved pathways were unclear. Since AD is characterized by the imbalance in Th1/Th2 ratio [35], there should be more studies on the inhibitory effects of O. indicum extract on relevant pro-inflammatory interleukins, cytokines or chemokines that mast cells and leukocytes release to control T cells derivation and functionality. The ethyl acetate fraction from O. indicum contained flavonoids with notable immunomodulatory activities including baicalein, oroxylin A and chrysin (preliminary study). These constituents took up large proportions in the extract and might play major roles leading to the results of this experiment.

5. CONCLUSION

The ethyl acetate fraction from O. indicum extract was effective in inhibiting DNCB-induced AD-like symptoms and exhibited its anti-inflammatory effect most apparently at 5% concentration. Although it is still unclear which phytocomponents have been accounted for the hindrance of this skin condition, high content of flavonoids in the extract, among which were oroxylin A, baicalein and chrysin, might be attributable. These presented results would hopefully encourage more studies to analyze the mechanism behind which O. indicum have exerted anti-inflammatory effects, as well as the possible synergistic interaction between present chemical constituents, for the purpose of
exploiting the potential of this valuable medicinal plant to the fullest.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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