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Evaluation the Effectiveness of Phenolic Compound of *Salvia frigida* on Induced Atopic Dermatitis in Experimental Mice

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Abstract

To evaluate the effectiveness of Phenolic Compound of *Salvia frigida* on induced atopic dermatitis (AD) of mice. Forty mice were included in the study, divided in to four groups (10 mice/group): apparently healthy, induced AD without treatment, induced AD treated with tacrolimus 0.1% ointment, and induced AD treated with Phenolic Compound of *Salvia frigida* cream 5%. Examination of histopathology was done and skin homogenates levels also measured. Levels of WBC, Eosinophil, skin tissue homogenate of IL-13 and IL-4, serum IgE, and histopathological scores were significantly increased among induced non treated AD group in comparison with the control group. Comparisons of non-treated induced AD group with *Salvia frigida* or Tacrolimus treated groups; shows a significant reduction in the levels of all studied parameters' (WBC, Eosinophil, skin tissue homogenate of IL4- and IL-13, serum IgE, observational severity score, and histopathological scores) after the application of Tacrolimus 0.1% ointment. While after the application of phenolic compound cream 5%, it shows a significant reduction in the levels of all parameters except those of (eosinophil, IgE, and IL-13). The comparison between the effect of topical application of tacrolimus and phenolic compound on the studied variables shows that the levels of epidermal thickness was significantly lower after application of phenolic compound among studied groups, while the levels of WBC and inflammatory cell were significantly lower after application of tacrolimus among studied groups. In conclusion, the use of these therapeutic agents that target IgE, IL-4 and IL-13 could be promising in the treatment of AD.

Keywords: Phenolic Compound, *Salvia frigida* , Atopic dermatitis, Tacrolimus, Interleukin-4, Interleukin-13

تقييم فعالية المركب الفينولي لسالفيا فريجيدا بالمقارنة مع تاكروليموس على التهاب الجلد التحسسي المستحث في الفئران المختبرية

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الخلاصة

لتقييم فعالية المركب الفينولي لسالفيا فريجيدا على التهاب الجلد التأتبي في الفئران المختبرية. تم تضمين أربعين فأرًا في الدراسة ، مقسمة إلى أربع مجموعات (١٠ فئران / مجموعة): صحية ، مُحفَّزة بالتهاب الجلد التأتبي دون علاج ، مُحفَّزة بالتهاب الجلد التأتبي مُعالج بتاكروليموس ٠,١٪ مرهم ، و مُحفَّزة بالتهاب الجلد التأتبي معالج بمركب الفينول من كريم سالفيا فريجيدا ٥٪ . تم إجراء فحص التشريح المرضي وقياس مستويات تجانس الجلد. وجد زيادة في مستويات كريات الدم البيضاء و الخلايا الحمضية و في الانترلوكين ١٣ و الانترلوكين٤ والاجسام المضادة أي في الدم ونتائج الأنسجة المرضية بشكل ملحوظ بين المجموعة المستحثة غير المعالجة بالمقارنة مع المجموعة الضابطة. مقارنات بين المجموعة المُحفَّزة بالتهاب الجلد التأتبي غير المعالجة مع المجموعات المعالجة بسالفيا فريجيدا أو تاكروليموس ؛ يُظهر انخفاضًا كبيرًا في مستويات جميع المعلمات المدروسة بعد وضع مرهم تاكروليماس. بينما يظهر بعد تطبيق الكريم المركب الفينولي انخفاضًا ذات دلالة احصائية في مستويات جميع العوامل باستثناء تلك (الخلايا الحمضية، الانترلوكين٤ والاجسام المضادة أي) .أظهرت المقارنة بين تأثير التطبيق الموضعي للتاكروليموس والمركب الفينولي على المتغيرات المدروسة أن مستويات سماكة البشرة كانت أقل بشكل ملحوظ بعد تطبيق المركب الفينول بين المجموعات المدروسة، بينما كانت مستويات كريات الدم البيضاء والخلية الالتهابية أقل بشكل ملحوظ بعد تطبيق عقار تاكروليموس بين المجموعات المدروسة. من المحتمل أن يكون استخدام هذه العوامل العلاجية التي تستهدفا لانترلوكين ١٣ و الانترلوكين ٤ والاجسام المضادة أي مفيدًا في علاج التهاب الجلد التأتبي.

الكلمات الأساسية: مركب الفينول ، سالفيا فريجيدا ، التهاب الجلد التأتبي ، تاكروليموس ، إنترلوكين ٤ ، إنترلوكين ١٣

Introduction

Atopic dermatitis (AD) also known as atopic eczema, it is a common familial chronic inflammatory skin disease, determined by xerosis (increased water loss through the skin), itching, scaly and erythematous skin lesions, and high serum levels of IgE. Between 10 to 20% of children and 1 to 3% of adults worldwide affected by it and has negative medical and social effect on patients and their families. About 85% of affected children develop the disease before the age of 5 years (60% before the age of 1). Patients may get off of this condition (improvement during puberty is a common phenomenon). It may persist or appear for the first time into adulthood [(1)].

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Individuals with AD have frequent and sometimes severe bacterial and viral infections Skin infections. Herpetic eczema (caused by the herpes simplex virus) It is known to occur mainly in AD patients. Nearly 80-100% of patients have AD disease Colonization of *Staphylococcus* aureus bacteria on their skin (which often forms From a heterogeneous mixture), compared to only 5-30% of the normal population [(2)].

AD treatment should be geared towards restoring the skin barrier which includes moisturizing and repairing the skin, reducing itching and reducing inflammation when necessary. Therefore, the successful treatment of AD requires a polymorphic approach that involves patient and caregiver education, optimal skin care practices, anti-inflammatory treatment with topical corticosteroids and/or topical Calcineurin inhibitors, and skin infections treatment [(3)]

Tacrolimus is the generic name for the macrolide immunosuppressant formerly known by its experimental name FK506. Tacrolimus was the first discovered while examining for activity of antibacterial of a multitude compound. This macrolide is produced by Streptomyces tsukubaensis, a bacterium found in the soil near Tsukuba, Japan**.** [(4)] It shows a good penetration through the skin due to its small size (molecular weight 822) and can be used to improve the severity of AD through its immune regulation and improve control of acute attacks and prevention of new ones due its mechanism of action as immune regulation. [(5, 6).] Tacrolimus has side effects, such as skin burning and itching [(7)]. Accordingly, effective therapy with fewer side effects is required for treatment of AD. The World Health Organization encourages, promotes and facilitates effective herbal health programs [(8)]. Salvia plant is the largest genus of the Lamiaceae family. It has around 1000 species distributed over the world [(9)]. *Salvia frigida* is one of the most medicinal plants that is used frequently in Turkey [(10)] .

The acetone extract of the aerial parts of *Salvia frigida* has been tested previously. Two oleanane type (Erythrodiol, Olean-12-ene-3β-ol) and two cycloartane type triterpenoids (24-Methylenecycloartanol, Cycloartanol) with the compounds α –amyrin, and β –sitoserol were isolated and identified [(11)] .

Pharmacological activity of *Salvia frigida* extract are: Xanthine oxidase inhibition, antioxidant activities [(12)], anticholinesterase effect [(13)], and anticancer activities [(14)].

Plant phenolic compounds (PCs) are biologically generated, secondary metabolites. It is found universally in the plant kingdom [(15)]. The antioxidant properties of PCs and flavonoids are thought to be mediated by scavenging free radicals such as ROS and RNS, and to suppress formation of ROS and RNS by inhibition specific enzymes or chelating trace minerals needed for their production, and finally by up regulating or protecting the antioxidant defense system [(16, 17)].

Although the currently used medications in the treatment of AD are effective in managing the disease; adverse reactions may decrease their usefulness [(7)]. Accordingly, the present study was designed to evaluate the effectiveness of phenolic compound of *Salvia frigida* on induced atopic dermatitis mice model through their effect on WBC, Eosinophil, serum IgE, tissue homogenate of IL4 and IL13, observational severity score, and histopathological score. The study also aimed to compare the anti-inflammatory effect of phenolic compound of *Salvia frigida* with Tacrolimus on induced atopic dermatitis mice model.

Materials and Methods

A randomized prospective, controlled animal study was carried out. This study was conducted from 1st of November 2020 to 30th of April 2021, in the Department of pharmacology-College of Medicine-Al Nahrain University. The protocols for the animal experiment used were carefully reviewed for ethical and scientific care procedures and approved by Al- Nahrain University – College of Medicine review Council (Approval Number 857 in 28/9/2020).

Experimental design and animal groups

A total of 40 healthy adult male Albino mice (25-30g) collected from the animal house. The mice were housed in animal house in a good ventilated isolated place; with a room temperature of 20-24°C. The animals were left for seven days to acclimatize to the animal room conditions and allowed free access to water and Ad libitum feeding. The animals were housed in animal house, at College of Veterinary Medicine in a good ventilated isolated place; with a room temperature of 20-24°C, and kept light for 12 hours. The practical part of the study was directed at College of Veterinary Medicine, University of Baghdad, Baghdad- Iraq.

Ten mice were chosen randomly and considered as a healthy control group and compared with other induced groups. Thirty mice treated with 1-Chloro-2, 4-dinitrobenzene (DNCB) induced AD and randomly divided into three groups 10 mice/group: (induced AD mice non treated, induced AD mice treated with Tacrolimus 0.1% ointment, and induced AD mice treated with Phenolic Compound of *Salvia frigida* cream ٥% topically). Topical treatment was applied once daily at 9:00 AM for 21 days.

Induction

Mouse model of DNCB-induced atopic dermatitis

Mice described AD skin through shaving hair from dorsal of skin then 150 µL of 1% DNCB in 3:1 (v/v) acetone/olive oil solution was topically applied once to the exposed skin. Five days after dorsal hair removal, 0.2% DNCB dissolved in an

acetone: olive oil mixture (3:1 vol/vol) was applied to challenge the dorsal skin (150 µL) three times a week for 3 weeks. After the visual confirmation of skin sensitization, mice were treated with test samples. (18) Figure 1.

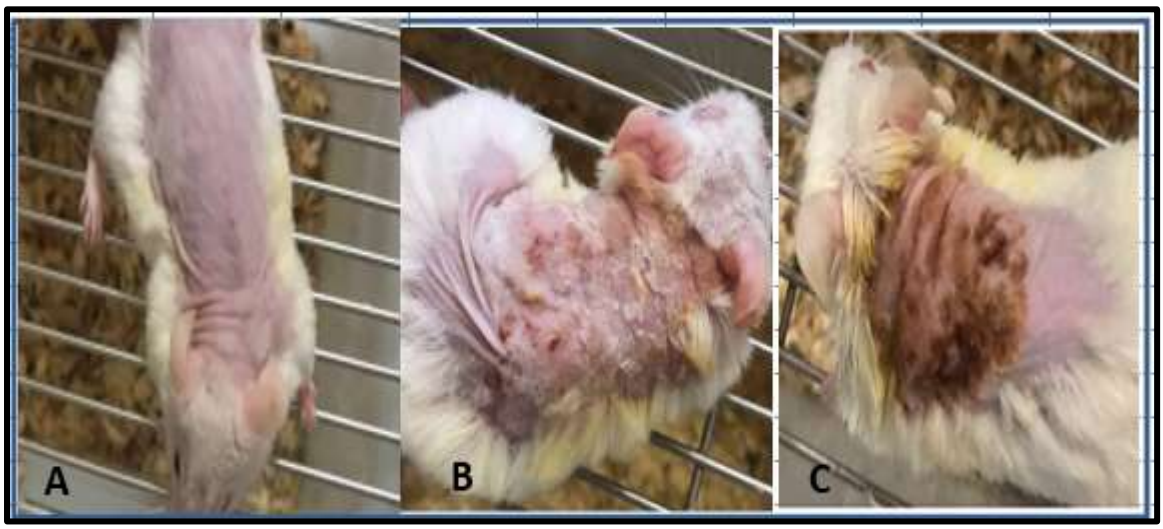

Figure 1. Normal skin lesion without induction (A), Induced atopic dermatitis skin lesion (B) (C).

Plant material

The aerial parts of *Salvia frigida* were extracted and authenticated in November, 2020 by Department of Pharmacognocy and medicinal plants / College of Pharmacy/ Al-Mustasryiah University (Iraq).

Salvia frigida extraction*:*

150 gm of shade- dried pulverized leaves was defatted by maceration with hexane for 24 h then allowed to dry at room temperature. The defatted plant materials were extracted using Soxhlet apparatus in which the powder packed in the thimbles and extracted with 1.75 L of aqueous methanol 85% as a solvent extraction for 24 hours. The extract was filtered and the solvent was evaporated under reduced pressure using a rotary evaporator to get 12 gm dry extract. 4 gm from the residual was suspended in 100ml water; about 3-4 ml of 5% sodium hydroxide was added to obtain a basic solution having PH 10 and partitioned with ethyl acetate (3*100 ml) (19, 20). The aqueous layer collected and evaporated to dryness which represents the phenolic compounds rich fraction that was used.

Preparation of phenolic compound 5% cream

5 gm of phenolic compound extracted from *Salvia frigida* was weighted and dissolved in 3 ml of alcohol and shaking it for 4 minutes until it dissolved completely and became clear, after that we complete the weight to 100 gram with aquasoft cream (Ajanta Company) and shake the combination for 5 minutes by spatula.[21]

High Performance Liquid Chromatography (HPLC) for quantitative and qualitative detection plant extractions

The identification was made by (HPLC**)** by comparing the retention times obtained at identical chromatographic conditions between extract fraction and standards. The concentration for the extraction of phenolic compound was quantitative determined by comparing the peak area of the standard with of the sample [22] .

Treatment protocols

The topical applications of treatments (Tacrolimus 0.1% ointment [23] and Phenolic compound of *Salvia frigida* 5% cream)[24] were applied to atopic dermatitis area of animal for 21 days once daily at 9 AM starting from the fifth day of induction.

Parameters of study

The following parameters are used to compare the results between experimental groups after day 21 of treatment: WBC and Eosinophil count, Serum IgE, IL-4 and IL-13 were measured in skin tissue homogenate for mice with atopic dermatitis skin lesion, histopathological evaluation of atopic dermatitis skin lesion and compared with those of controls, and assessment of observational severity score.

Animal sacrificing, dissection, histological analysis, skin tissue homogenate preparation, and assessment of observational severity score

At the 21th day of the treatment, we took the whole number of mice from each study groups and anesthetized through a piece of cotton socked with ether put with the mouse inside a closed jar for few minutes to ensure be anesthetized by inhalation,

blood sample collected (1ml) in EDTA tube for CBC and serum IgE, then sacrificed by cervical dislocation and atopic dermatitis skin area was cut by sharp blade; this skin wound was dissection into two equal pieces one for the histological analysis and the second for the preparation of skin homogenate. The remaining mice from each group were subjected to the same procedure at the 21th day of the treatment.

Histological section preparation

Dorsal skin samples were collected from each animal in study groups and fixed in 10% formaldehyde paraffin embedded and cut into 6 µm sections. Deparaffinized sections were stained with ordinary hematoxylin and eosin (H&E) to determine inflammatory degree and histological changes associated with atopic dermatitis [(25)] .

Assessment of histopathological changes of skin sections

Histopathological follow-up procedures were used for the skin samples taken from each group on the 21 days of treatment. Histopathological changes of skin of each specimen were evaluated and scored by semi quantitative scoring systems for the evaluation of mouse model histopathology include epidermal hypertrophy, hyperkeratosis, parakeratosis, erosion, inflammatory cell infiltration, and extracellular edema, each scored from 0 to 3 (0 no abnormality, 1+ slight, 2+ mild, and 3+ moderate)[(26)], has been examined by pathologist and carried out in histopathology department /Ibn Sina University of Medical and Pharmaceutical Sciences to observe the changes in tissues.

Skin tissue homogenate preparation

The second piece of skin obtained were washed with normal saline, and rinsed with chilled phosphate buffer saline (1X PBS), put with filter paper and weighed. Each 100 mg of skin wound tissue was homogenized with 1 ml of (1X PBS) with the aid of tissue homogenizer [(27)] for 1 minute at 4 °C, and must be stored overnight at 20°C. Two freeze-thaw cycles must be performed to break the cell membranes; the homogenates were centrifuged for ten minutes at 2000 RPM at 2-8 °C. The supernatant was obtained and stored at –20°C to the assay of IL-4 and IL-13 levels in the tissue.

The quantitative measurement of IgE, IL-4 and IL-13 (principle of the assay)

Serum IgE: (The enzyme-linked immunosorbent assay).

ELISA Kit for the estimation of IgE was obtained from CUSABIO\China Kit. Specific different antibodies can be measured quantitatively by the enzyme-linked immunosorbent assay (ELISA). After incubating the tested serum in an antigen-coated polystyrene plat or tube, enzyme specifically labeled anti-immunoglobulin is then added and the remaining in the plate after washing will give a measure to the quantity of specifically related antibody in the serum. The procedure depends on the insolubilization of specific antigens by passive adsorption to a solid phase (plate), example polystyrene phase [(28)]. The procedure is done according to the manufacturer's instructions.

Skin tissue homogenate of IL-4 and IL-13

ELISA Kit for the estimation of IL-4 and IL-13 was obtained from CUSABIO\China Kits was established on the base of sandwich enzyme-linked immunosorbent assay technology. Anti- IL-4 and Anti- IL-13 antibodies were precoated onto 48-well plates. And as detection antibodies, the biotin conjugated Anti- IL-4 and anti- IL-13 antibodies were used. We added; the standards, test samples and biotin conjugated detection antibodies to the wells subsequently, and washed with wash buffer. HRP-Streptavidin was added and unbound conjugates were washed away with wash buffer.

To visualize HRP (horseradish peroxidase) enzymatic reaction TMB (3, 3′, 5,5′-Tetramethylbenzidine) substrates were used. TMB (3, 3′,5,5′-Tetramethylbenzidine) was catalyzed by HRP to produce a blue color product which changed into yellow in accordance to adding acidic stop solution. The density of yellow color is proportional to amount of IL-4 and IL-13 of the sample captured in plate. We read the optical density absorbance at 450nm in a microplate reader, and then the concentration of IL-4 and IL-13 was calculated by comparing the optical density of the samples to that of standard in the corresponding microtiter plate. The concentration of IL-4 and IL-13 in each sample was expressed in pg/ml for comparison of the results with those of controls concentration [(29)] .

Assessment of observational severity score

The severity of AD on the dorsal area was evaluated for each group on the 21th days of treatment. The evaluation of erythema, dryness, erosion and edema scored as 0 (none), 1 (mild), 2 (moderate), and 3 (severe). Clinical skin score was defined as the summation of each individual scores, range from 0 to 12 [(30)] .

Statistical analysis

Data of the study were collected, analyzed, and presented using Microsoft Office Excel 2010 and statistical package for the social sciences SPSS software version 23. Numeric variables were expressed as mean ± SD and all statistical comparisons were made by means of independent t-test and ANOVA test. When $P \leq 0.05$ was considered statistically significant, and highly significant when $P \leq 0.01$. The correlation was done between observational severity score, IL-4, and IL-13 using Pearson correlation test.

Results

Comparison between control and non-treated atopic dermatitis induced group regarding WBC, Eosinophil, serum IgE, skin tissue homogenate of IL-13 and IL-4, histopathological scores, and observational severity score:

Inflammatory signs have been seen from the first day in all induced non treated group. The levels of WBC, eosinophil, skin tissue homogenate of IL-13 and IL-4, and serum IgE, were significantly increased among induced non treated atopic dermatitis group in comparison with control group (P=0.01, P=0.001, P=0.004, P<0.001 and P<0.001 respectively). Table 1, Figure 2 .

Table 1. Comparison between controls and non-treated atopic dermatitis induced group regarding WBC, Eosinophil, serum IgE, and skin tissue homogenate of IL-13 and IL-4:

Variables	Mean ±SD Non-treated group	Mean ±SD Control group	P* value
WBC (x103 /µl)	10±2.1	3.3 ± 2	0.01
Eosinophil (x103 /µl)	2.5±0.02	0.0±0.0	0.001
IgE(ng/ml)	26.62±5.15	15.59±8.65	0.004
IL13 (pg/ml)	57.8±105.29	22.303±68.76	<0.001
IL4 (pg/ml)	22.11±6.21	6.68±3.01	<0.001
*Independent sample t test where p significant at ≤ 0.05 and high significant at <0.001			

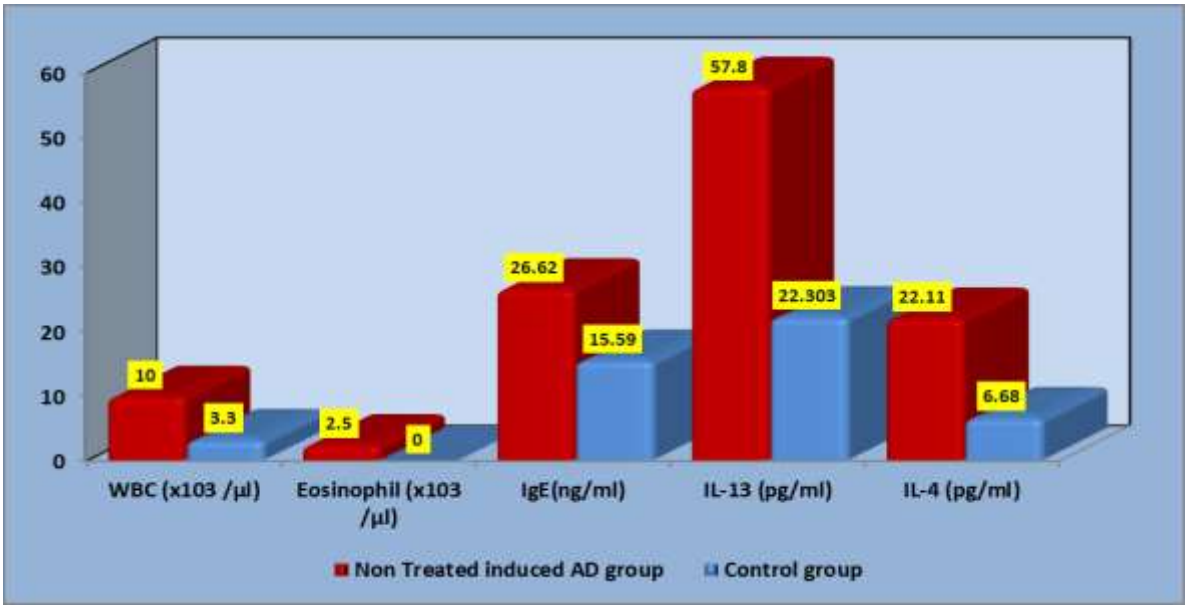

Figure 2. Comparison between means of controls and non-treated atopic dermatitis induced group regarding WBC, Eosinophil, serum IgE, skin tissue homogenate of IL-13 and IL-4.; AD: atopic dermatitis. WBC: white blood cells, IgE: immunoglobulin E, IL-13: interleukin 13, IL-4: interleukin 4. Results are expressed as mean ± SD, P is significant at ≤ 0.05

Observational severity score and histopathological changes showed a significant high elevation among induced non treated group than among controls, P<0.01. Table 2, Figure 3.

Figure 4 shows the histopathological changes in topically induced AD group in comparison with controls.

Table 2. Comparison between controls and non-treated atopic dermatitis induced group regarding histopathological scores, and observational severity score.

Variables	Mean ±SD Non-treated group	Mean ±SD Control group	P* value
Epidermal Thickness	3.50±0.52	0.0±0.0	<0.001
Hyperkeratosis	3.00±0.81	0.0±0.0	<0.001
Parakeratosis	3.40±0.69	0.0±0.0	<0.001
Erosion	1.50±0.52	0.0±0.0	<0.001
Inflammatory Cell	2.60±0.51	0.0±0.0	<0.001
Extracellular Edema	2.50±0.52	0.0±0.0	<0.001
observational severity score	10.00±.81	0.0±0.0	<0.001
***Independent sample t test where p significant at ≤ 0.05 and high significant at <0.001**			

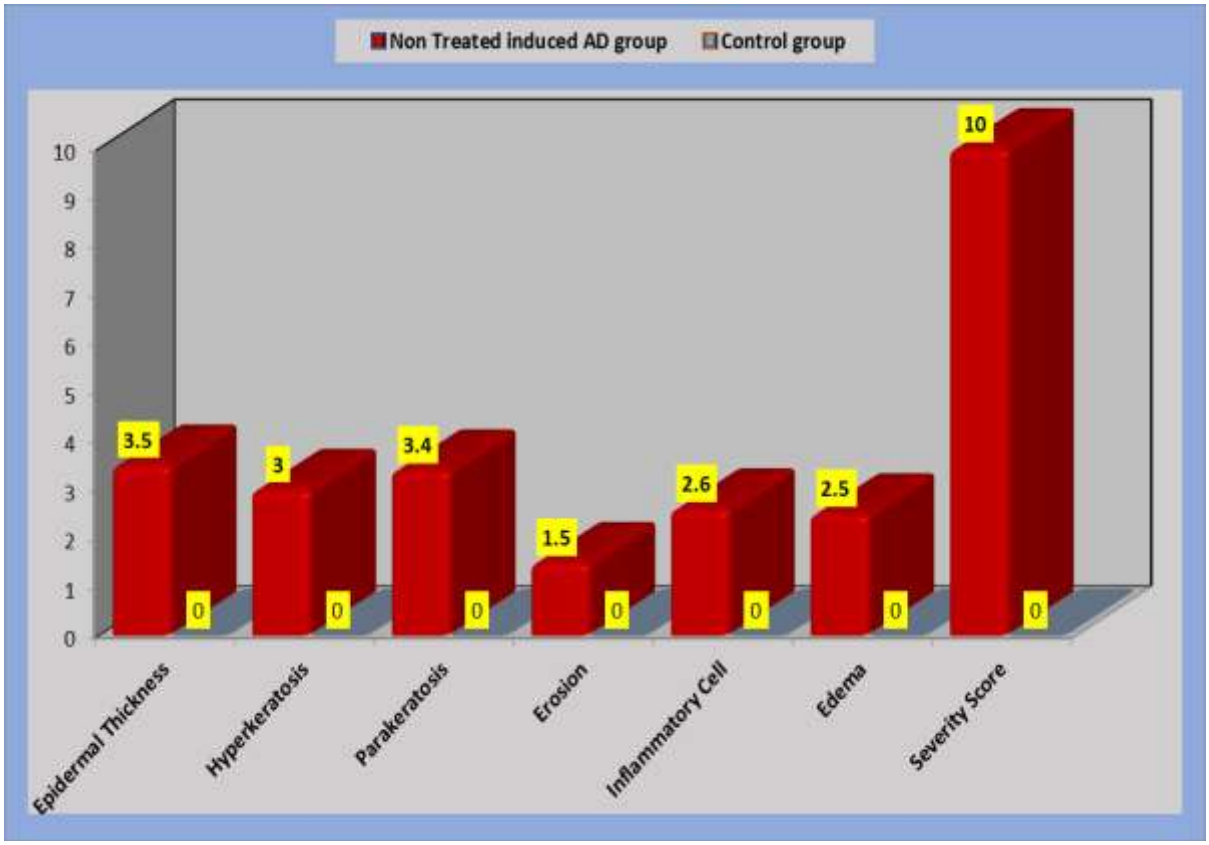

Figure 3. Comparison between means of controls and non-treated atopic dermatitis induced group regarding histopathological scores and Observational severity score; AD: atopic dermatitis; Results are expressed as mean ± SD, P is significant at ≤ 0.05

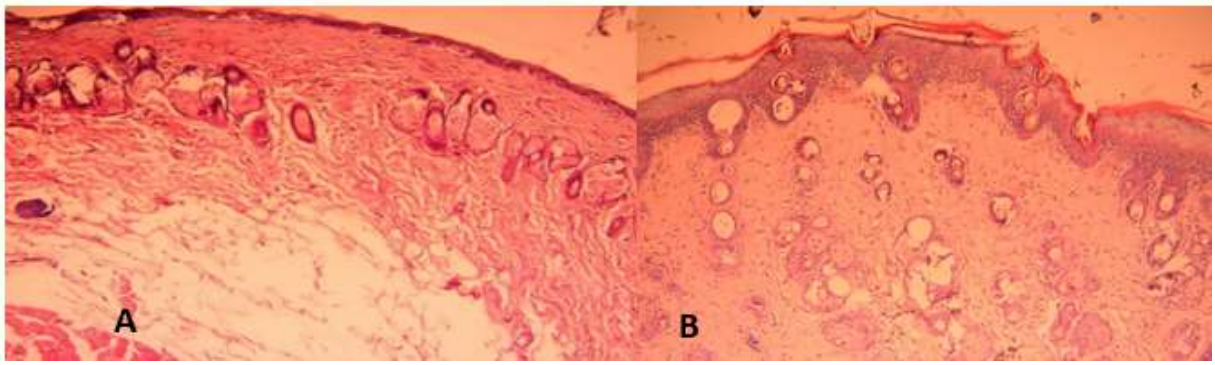

Figure 4. Histopathological changes in topically induced AD group (B) in comparison with controls (A) (10x): ordinary Hematoxylin and eosin stain.

Comparisons of non-treated atopic dermatitis induced group with each of (Salvia frigida treated group and Tacrolimus treated group); regarding WBC, Eosinophil, serum IgE, and skin tissue homogenate of IL-4 and IL-13, observational severity score, and histopathological score:

According to the comparison between non-treated atopic dermatitis induced group and phenolic compound of *Salvia frigida* treated group; skin tissue homogenate of IL4 and WBC were affected by the treatment with phenolic compound of *Salvia frigida* 5% cream topically which appear clearly in the result tabulated in table (3) that variables were significantly decreased from those of non-treated induced AD group (P=0.002, and P=0.042 respectively) Table 3.

A significant improvement in the histopathological parameters and in the observational severity score after treatment with topical 5% phenolic compound of *Salvia frigida* was observed compared with atopic dermatitis induced group. Table 3, Figure. 5.

Table 3. Comparisons of non-treated atopic dermatitis induced group with phenolic compound of *Salvia frigida* treated group regarding WBC, Eosinophil, serum IgE, and skin tissue homogenate of IL-4 and IL-13, observational severity score, and histopathological score.

Variables	Mean±SD Non Treated	Mean±SD *Salvia*	P*
WBC (x103 /µl)	10±2.1	7.6± 3.03	0.042
Eosinophil (x103 /µl)	0.05± 0.06	0.03±0.03	0.166
IgE(ng/ml)	26.62±5.150	20.36±5.92	0.32
IL13 (pg/ml)	57.776±10.529	37.244±18.002	0.06
IL4 (pg/ml)	22.11±6.21	11.59±2.23	0.002
Epidermal Thickness	3.50±0.52	1.00±0.66	0.001
Hyperkeratosis	3.00±0.81	1.60±0.51	<0.001
Parakeratosis	3.40±0.69	1.20±0.78	<0.001
Erosion	1.50±0.52	0.40±0.51	<0.001
Inflammatory Cell	2.60±0.51	1.80±0.78	0.015
Extracellular Edema	2.50±0.52	1.10±0.56	<0.001
Observational Severity Score	10.00±0.81	3.70±1.33	<0.001
*Independent sample t test where p significant at ≤ 0.05 AD: atopic dermatitis			

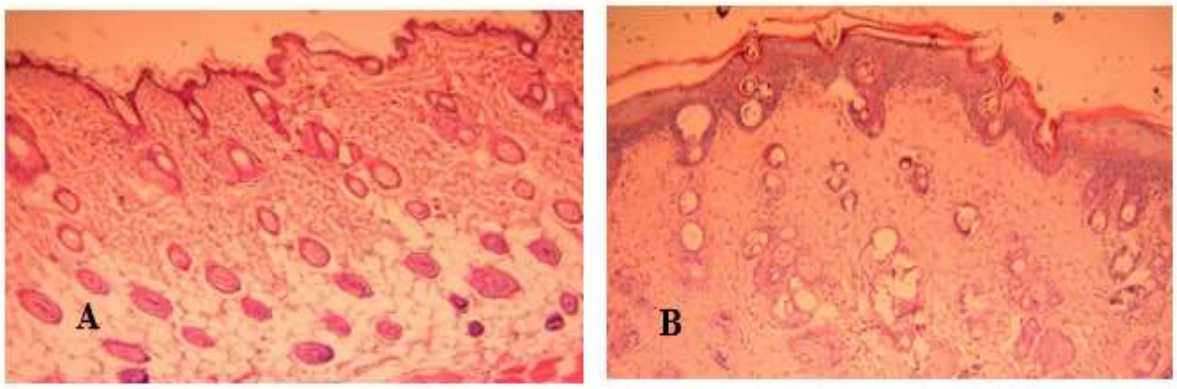

Figure 5. Histopathological changes in topically AD induced group (B) in comparison with phenolic compound of *Salvia frigida* treated group (A) (10x): ordinary Hematoxylin and eosin stain.

Comparisons of non-treated atopic dermatitis induced group with Tacrolimus treated group; regarding WBC, Eosinophil, serum IgE, and skin tissue homogenate of IL-4 and IL-13, observational severity score, and histopathological score:

Comparison between topically Tacrolimus treated group and non-treated induced atopic dermatitis group postulated in table 4 which elucidate clearly that the levels of WBC, Eosinophil, serum IgE, IL-13 and IL-4 in mice received Tacrolimus 0.1% ointment topically were significantly lower than the corresponding levels in AD induced non-treated group, (P=0.02, P=0.013, p=0.022, p=0.025 and P<0.001 respectively). Table 4.

Observational severity score and histopathological changes (epidermal thickness, hyperkeratosis, parakeratosis, erosion, inflammatory cell infiltrate, and extracellular edema) were significantly reduced in Tacrolimus treated group in comparison with those non-treated AD induced group, P<0.001. Table 4, Fig. 6.

Table 4. Comparisons of non-treated atopic dermatitis induced group with Tacrolimus treated group regarding WBC, Eosinophil, serum IgE, and skin tissue homogenate of IL-4 and IL-13, observational severity score, and histopathological score.

Variables	Mean±SD Non Treated	Mean±SD Tacrolimus	P*
WBC (x103 /µl)	**10±2.1**	**6.03± 2.02**	**0.02**
Eosinophil (x103 /µl)	**0.05± 0.07**	**0.020± 2.02**	**0.013**
IgE(ng/ml)	**26.62±5.150**	**16.0±6.08**	**0.022**
IL13 (pg/ml)	**57.776±10.529**	**31.82±21.3**	**0.025**
IL4 (pg/ml)	**22.11±6.21**	**9.05±4.03**	**<0.001**
Epidermal Thickness	**3.50±0.52**	**1.20±1.22**	**<0.001**
Hyperkeratosis	**3.00±0.81**	**1.60±0.51**	**<0.001**
Parakeratosis	**3.40±0.69**	**1.20±0.78**	**<0.001**
Erosion	**1.50±0.52**	**0.20±0.42**	**<0.001**
Inflammatory Cell	**2.60±0.51**	**1.70±0.42**	**0.001**
Extracellular Edema	**2.50±0.52**	**1.20±0.51**	**<0.001**
Observational Severity Score	**10.00±0.81**	**4.50±1.08**	**<0.001**

*Independent sample t test where p significant at ≤ 0.05
AD: atopic dermatitis

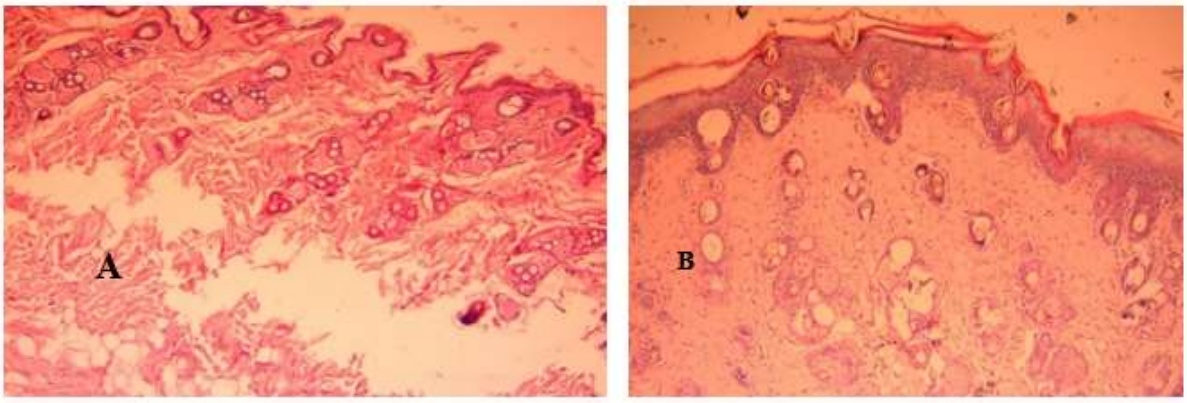

Figure 6. Histopathological changes in topically AD induced group (B) in comparison with Tacrolimus treated group (A) (10x): ordinary Hematoxylin and eosin stain.

The Comparison between three groups (phenolic compound of Salvia frigida , Tacrolimus treated groups, and non-treated atopic dermatitis induced group regarding WBC, Eosinophil, serum IgE, and skin tissue homogenate of IL-4 and IL-13, histopathological changes and score

In comparison between the effect of topical Tacrolimus and phenolic compound on the studied variables, the level of epidermal thickness was significantly lower after phenolic compound of *Salvia frigida* treatment among studied groups (P=0.025). The level of WBC and inflammatory cell were significantly lower after tacrolimus treatment among studied groups (P=0.04 and P=0.046 respectively). Reduction of erosion was more significant among Tacrolimus treated groups, P<0.001. Table 5, Figure (7, 8, 9)

Table 5. Comparison between non treated atopic dermatitis induced group with each of *Salvia frigida* and Tacrolimus treated groups (by one way ANOVA test) regarding WBC, Eosinophil, serum IgE, and skin tissue homogenate of IL-4 and IL-13.

Variables	Mean±SD Salvia group	Mean±SD Tacrolimus group	Mean±SD Non-treated	P
WBC (x103 /µl)	7.6± 3.03	6.03± 2.02	10±2.1	0.04
Eosinophil (x103 /µl)	0.03±0.03	0.020± 2.02	1± 0.07	<0.001
IgE(ng/ml)	20.36±5.92	16.0±6.08	26.62±5.150	0.029
IL13 (pg/ml)	37.24±18.0	31.82±21.3	57.8±10.529	0.022
IL4 (pg/ml)	11.59±2.23	9.05±4.03	22.11±6.21	<0.001
Epidermal Thickness	1.00±0.66	1.20±1.22	3.50±0.52	0.025
Hyperkeratosis	1.60±0.51	1.60±0.51	3.00±0.81	<0.001
Parakeratosis	1.20±0.78	1.20±0.78	3.40±0.69	<0.001
Erosion	0.40±0.51	0.20±0.42	1.50±0.52	<0.001
Inflammatory Cell	1.80±0.78	1.70±0.42	2.60±0.51	0.046
Extracellular Edema	1.10±0.56	1.20±0.51	2.50±0.52	<0.001
Observational Severity Score	3.70±1.33	4.50±1.08	10.00±0.81	<0.001

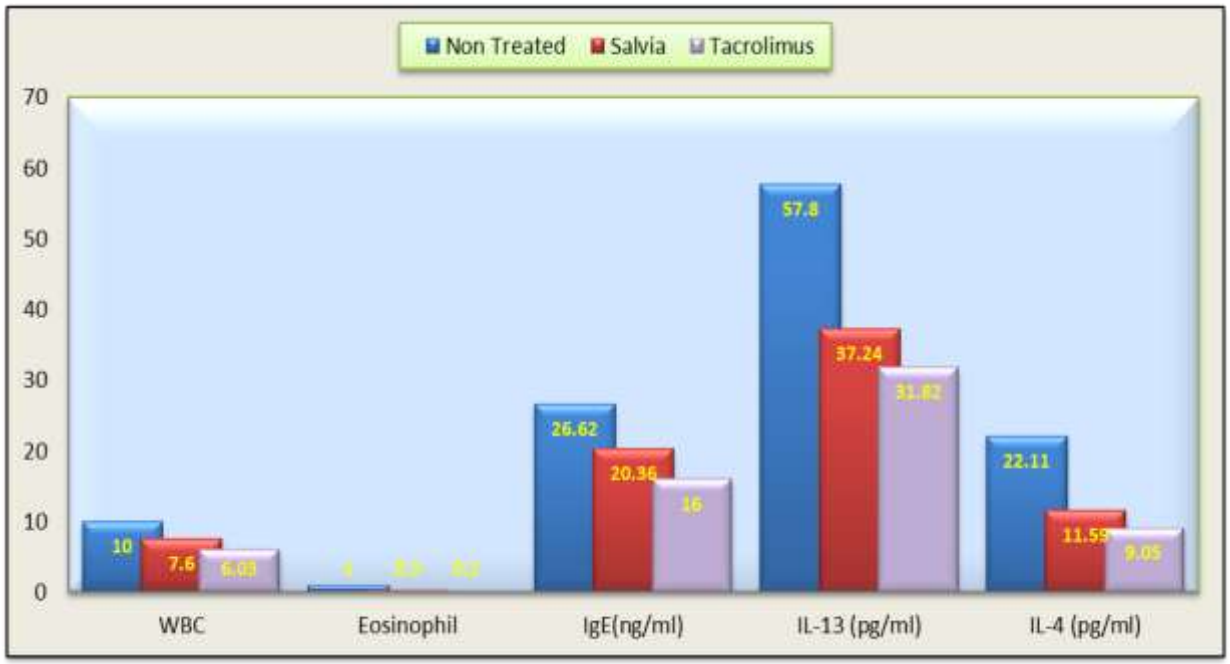

Figure 7. Comparison between non treated atopic dermatitis induced group with each of *Salvia frigida* and Tacrolimus treated groups (by one way ANOVA test) regarding WBC, Eosinophil, serum IgE, and skin tissue homogenate of IL-4 and IL-13. WBC: white blood cells, IgE: immunoglobulin E, IL-13: interleukin 13, IL-4: interleukin 4. Results are expressed as mean ± SD, P is significant at ≤ 0.05.

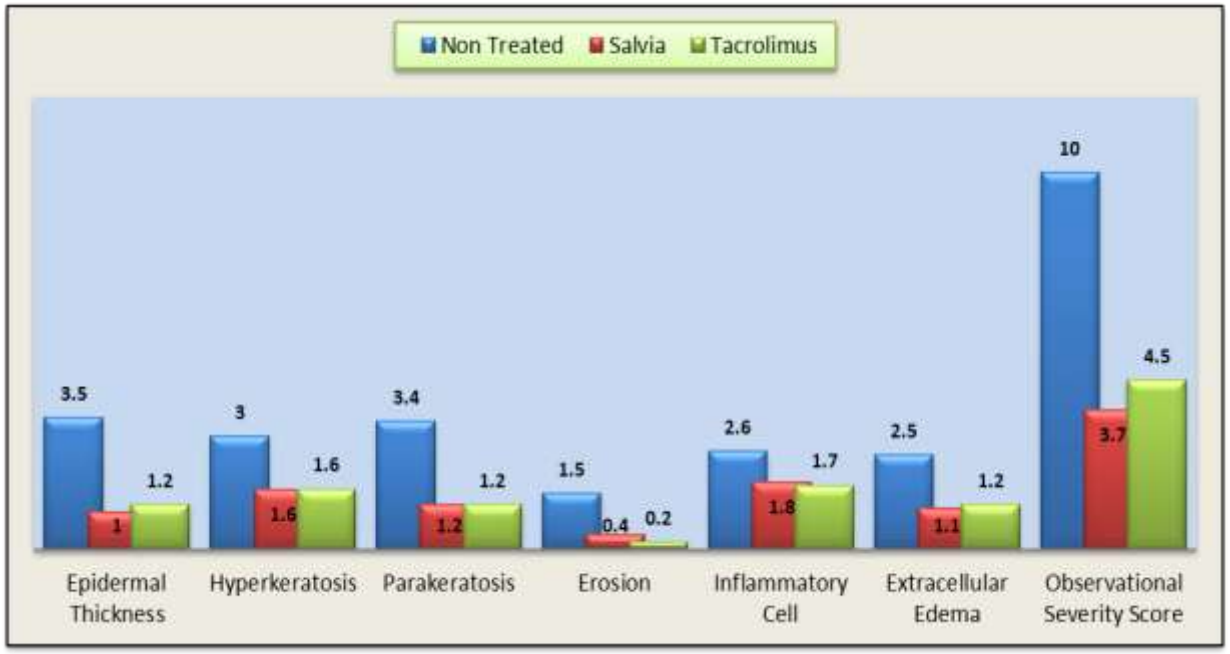

Figure 8. Comparison between phenolic compound of *Salvia frigida* and Tacrolimus treated groups (by one way ANOVA test) regarding histopathological changes and observational severity score. Results are expressed as mean ± SD, P is significant at ≤ 0.05.

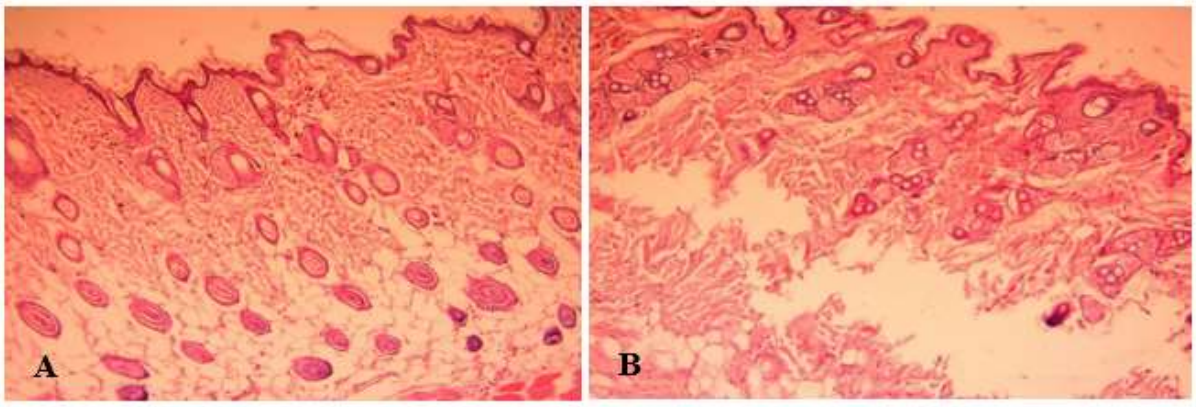

Figure 9. Comparison between phenolic compound of *Salvia frigida* treated group (A) and Tacrolimus treated group (B) (10x): ordinary Hematoxylin and eosin stain.

Correlations between observational severity score and IgE, IL-4 and IL-13 in all studied groups

Table 6 revealed that the levels of IgE, IL-4, and IL-13 were correlated positively and significantly with observational score of mice subjected to the present study. P<0.05

Table 6. Correlations between Observational severity score, IgE, IL-4 and IL-13 in all studied groups.

		IL13	IL4	Observational Severity Score
IgE	r	.532	.476	.393
	P Value	.002**	.008**	.032*
IL13	r		.440	.278
	P Value		.015*	.013*
IL4	r			.680
	P Value			.000**
****. Correlation is significant at the 0.01 level (2-tailed).**				
***. Correlation is significant at the 0.05 level (2-tailed).**				

Discussion

According to the above results, Comparison between apparently healthy group and AD induced non-treated group shows significant inflammation signs, in addition to significant increase in thickness and in the level of observational severity score among AD induced non-treated group. Similar to this result, a study showed an increase in all types of WBC in AD induced non-treated group [31]

Signs of inflammation, histopathological changes, and observational severity score after application of 5% phenolic compound or 0.1% tacrolimus ointment topically, were significantly declined when compared with non-treated induced AD group. This indicates that the anti-atopic effect of phenolic compound of *salvia frigida* is similar to the effect of tacrolimus.

In consistent with that, some studies found that salvia plant has anti-inflammatory effect among AD treated group with phenolic compound [32, 33] Many other studies confirm these results, concluded that the properties of Salvia plant include anti-inflammatory, anticancer, anticholinesterase, antimicrobial, antimalarial and antioxidant [34, 35].

It has been reported that there was a significant improvement in overall quality of life had been obtained and maintained throughout a 4-week tacrolimus treatment study period, as well as the improvements in erythema, pruritus and sleeplessness [36].

When compare between *Salvia frigida* and Tacrolimus treated groups in the present study, Salvia treated group shows a significant reduction in epidermal thickness after 3 weeks of treatment when compare with Tacrolimus treated groups while Tacrolimus treated group shows more significant reduction in WBC count and inflammatory cells in comparison to others. The reduction of erosion was more pronounced among Tacrolimus treated groups. Similarly, a study revealed that the application of different topical treatment apart on the atopic dermatitis like skin lesions reduced the inflammatory response on damaged skin barrier, which is caused by foreign allergic substances such as DNCB, and suppress the elevation of blood concentrations of histamine [37, 38]

The levels of IgE, IL-4 and IL-13 were correlated positively and significantly with observational severity score of mice model in this study. This finding appeared to be consistent with

another one that reported a positive correlation among same parameters [39].

These results indicate that using of these therapeutic agents that targeting IgE, IL4, and IL13 will probably useful in treatment of atopic dermatitis.

Conclusion

Topical application of tacrolimus ointment or phenolic compound of *Salvia frigida* seems to be effective in the treatment of atopic dermatitis through their abilities to decrease WBC, eosinophil, serum IgE, skin tissue homogenate of IL4, and IL13; as well as improving histopathological picture and reducing observational severity score, with more effectiveness of Tacrolimus than of *Salvia frigida* in the treatment of atopic dermatitis. A significant positive correlation was observed between serum IgE, skin tissue homogenate IL4, IL13 and observational score for atopic dermatitis mice model. The use of phenolic compound of *Salvia frigida* that target IgE, IL4, and IL13 could be promising l in the treatment of atopic dermatitis.

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Conflicts of interest

The authors declare no conflicts of interest.

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