

Evaluation the Effect of Phytosterol Fraction of *Chenopodium Murale* in Comparison with Tacrolimus on Mice Induced Atopic Dermatitis

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Abstract

Atopic dermatitis (atopic eczema), is a common familial chronic inflammatory skin disease, determined by xerosis, 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. To evaluate the effectiveness of Phytosterol Fraction of *Chenopodium murale* on DNCB-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 Phytosterol Fraction of *Chenopodium murale* cream 3% topically. Examination of histopathology was done and skin homogenates levels also measured using Mann Whitney U test to determine mean \pm SD. 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 control group. Comparisons of non-treated induced AD group with *Chenopodium murale* or Tacrolimus treated groups; shows a significant reduction in the levels of all studied parameters' (WBC, Eosinophil, skin tissue homogenate of IL-4 and IL-13, serum IgE, observational severity score, and histopathological scores) after the application of Tacrolimus 0.1% ointment or *Chenopodium murale* cream 3% topically. The comparison between the effect of topical application of tacrolimus and Phytosterol Fraction on the studied variables shows that the level of WBC and thickness of epidermis and inflammatory cells were significantly lower after tacrolimus treatment, while high significant reduction was founded in parakeratosis and score of observational severity among *Chenopodium murale* treated group in comparison with Tacrolimus treated group. In conclusion, topical application of phytosterol fraction of *Chenopodium murale* seems to be effective in treatment of atopic dermatitis through their abilities to decrease WBC, eosinophil, s. IgE, skin tissue homogenate of IL4, and IL13; as well as improving histopathology picture and reducing observational severity score. The use of phytosterol fraction of *Chenopodium murale* that target IgE, IL4, and IL13 could be promising in the treatment of atopic dermatitis.

Key words: Phytosterol Fraction, *Chenopodium murale*, Atopic dermatitis, Tacrolimus, Interleukin-4, Interleukin-13

تقييم فعالية جزيئات الفايستيرول لكينوبوديوم ميوريل بالمقارنة مع تاكروليموس على التهاب الجلد التحسسي المستحث في الفئران المخبرية

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الخلاصة

التهاب الجلد التأتبي (الأكزيما التأتبية)، هو مرض جلدي التهابي مزمن عائلي شائع، يتسم بالجفاف، والحكة، احمرار الجلد وتقشره، وارتفاع مستوى الاجسام المضادة أي في الدم. ما بين 10 إلى 20% من الأطفال و 1 إلى 3% من البالغين في جميع أنحاء العالم يصابون به وله تأثير صحي واجتماعي سلبي على المرضى وعائلاتهم. لتقييم فعالية جزيئات الفايستيرول لكينوبوديوم ميوريل على التهاب الجلد التأتبي في الفئران المخبرية. تم تضمين أربعين فأراً في الدراسة، مقسمة إلى أربع مجموعات (10 فئران / مجموعة): صحية، مُحفزة بالتهاب الجلد التأتبي دون علاج، مُحفزة بالتهاب الجلد التأتبي مُعالج بتاكروليموس 0.1% مرهم، و مُحفزة بالتهاب الجلد التأتبي معالج بجزيئات الفايستيرول من كريم كينوبوديوم ميوريل 3%. تم إجراء فحص التشريح المرضي وقياس مستويات تجانس الجلد. وجد زيادة في مستويات كريات الدم البيضاء و الخلايا الحمضية و في الانترلوكين 4 و الانترلوكين 13 و الاجسام المضادة أي في الدم ونتائج الأنسجة المرضية بشكل ملحوظ بين المجموعة المستحثة غير المعالجة بالمقارنة مع المجموعة الضابطة. مقارنت بين المجموعة المُحفزة بالتهاب الجلد التأتبي غير المعالجة مع المجموعات المعالجة بكينوبوديوم ميوريل أو تاكروليموس؛ يُظهر انخفاضاً كبيراً في مستويات جميع المعلمات المدروسة بعد وضع كريم الفايستيرول او مرهم تاكروليموس. أظهرت المقارنة بين تأثير التطبيق الموضعي لعقار التاكروليموس و جزيئات الفايستيرول على المتغيرات المدروسة أن مستويات كريات الدم البيضاء و سماكة البشرة و الخلايا المضادة للالتهاب كانت أقل بشكل ملحوظ بعد التطبيق الموضعي للتاكروليموس بين المجموعات المدروسة. نستنتج من ذلك أن التطبيق الموضعي لجزيئات الفايستيرول لكينوبوديوم ميوريل فعال في علاج التهاب الجلد التأتبي من خلال قدرته على تقليل كريات الدم البيضاء و الخلايا الحمضية و الانترلوكين 4 و الاجسام المضادة أي في الدم؛ وكذلك تحسين سماكة البشرة وتقليل درجة شدة الملاحظة. لهذا، قد يكون استخدام جزيئات الفايستيرول لكينوبوديوم ميوريل واعداً في علاج التهاب الجلد التأتبي.

الكلمات المفتاحية: جزيئات الفايستيرول، كينوبوديوم ميوريل، التهاب الجلد التأتبي، تاكروليموس، إنترلوكين 4، إنترلوكين 13

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Introduction

Atopic dermatitis (AD) is an inflammatory skin condition with pruritus; erythema; dryness; and scaly skin. Asthma presented in 30% of patients with AD. It is tending to be chronic with relapsing and remission periods. Patients can be cured during puberty; or persist for long life. ^(1, 2) Bacterial and viral infections of skin may be a consequences to AD. Herpetic eczema due to herpes simplex virus is frequently observed among AD patients. Most of patients with AD have Staphylococcus aureus infection. ⁽³⁾ Restoring skin barrier is the gold standard in the management of AD, often through skin moisturizing, decrease itching and inflammation. ⁽⁴⁾

Tacrolimus, a macrolide lactone of fungal origin (*Streptomyces tsukubaensis*), is an immunosuppressive drug commonly used in humans. Tacrolimus has been shown to inhibit the granules release of preformed mediators from skin mast cells and basophils and to down regulate the expression of IgE by mast cells, basophils, and Langerhan cells.⁽⁵⁾ Its small size make its ability to penetrate skin more powerful; therefore, it can be used in severe cases of AD and improve control of acute attacks and prevention of new ones due its mechanism of action as immune regulation.^(6, 7) Tacrolimus has side effects, such as skin burning and itching. ⁽⁸⁾ 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.⁽⁹⁾

Extracts from the leaves of various plants have various pharmacological activities, among these antioxidant activities due to their redox properties, which permit them to act as reducing agents, hydrogen donors, and single oxygen quenchers. The crude extract of various plants and its fractions were examined previously against different human pathogens including, *Escherichia coli*, *Klebsiella pneumonia*, *Bacillus subtilis*, and *Salmonella typhus*, by agar well diffusion method (anti-bacterial activity). Phytosterol fraction seemed to be a potential nutraceutical tool for some diseases such as gastrointestinal inflammatory disease. In addition, combining metabolic systematic and local anti-inflammatory effects (anti-inflammatory activity). Previous research showed that plant also useful as an anthelmintic, stomachic, antispasmodic, diaphoretic, sweaty, for amenorrhea pain, for abortion and for the relief of asthma, cold, and migraine. ⁽¹⁰⁻¹²⁾

Although the currently used medications in the treatment of AD are effective in managing the disease; adverse reactions may decrease their usefulness ⁽⁸⁾. Pharmacological activities of *Chenopodium murale*: antioxidant activity, anti-bacterial activity, anti-inflammatory and skin disease, Previous research showed that plant also

useful as an anthelmintic, stomachic, antispasmodic, diaphoretic, for amenorrhea pain, for abortion and for the relief of asthma, cold, and migraine ^(11, 13)

This study was carried out to evaluate the effectiveness of Phytosterol Fraction of leafs of *Chenopodium murale* on treatment of 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 Phytosterol Fraction of *Chenopodium murale* 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 December 2020 - June 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 animals and design of study

This study included 40 healthy adult male Albino mice weighted 25-30g. They 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, 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 (14) and randomly divided into three groups 10 mice/group (without using anesthetic medication in the AD induction period) ⁽¹⁵⁻¹⁷⁾. Induced AD mice non treated (negative control), induced AD mice treated with Tacrolimus 0.1% ointment (positive control), and induced AD mice treated with Phytosterol Friction of *Chenopodium Murale* cream 3% topically (test compound).⁽¹⁸⁾ Topical treatment was applied once daily at 9:00 AM for 21 days

Induction

Mouse model of 1-Chloro-2, 4-dinitrobenzene - 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, then after four days, 0.2% DNCB dissolved in an acetone: olive oil mixture (3:1 vol/vol) was applied to the same dorsal skin (150 µL) three times a week for 3 weeks. After

the visual confirmation of skin sensitization, mice were treated with test samples. ⁽¹⁴⁾ Figure 1.

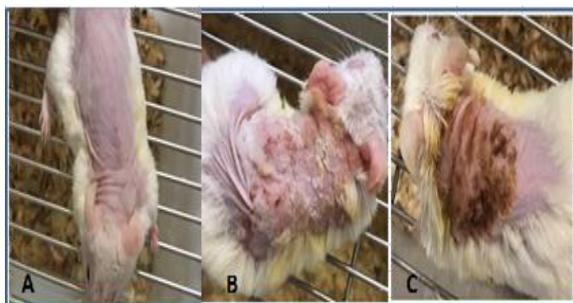


Figure 1. Normal skin lesion without induction (A), Induced atopic dermatitis skin lesion (B) and (C).

Plant material

Chenopodium murale plant was identified and authenticated by Prof. Dr. Ibrahim S. AlJubori /Department of Pharmacognocny/ College of Pharmacy/ Al-Mustansryiah University .The extraction of herb was executed in Pharmacognocny department, collage of Pharmacy, Al-Mustansryiah University (Iraq). Leaves were washed thoroughly, dried under shade, and ground in a mechanical grinder to coarsely powder.

The aerial parts of *Chenopodium murale* were extracted and authenticated in November, 2020 by Department of Pharmacognocny and medicinal plants / College of Pharmacy/ Al-Mustansiriya University (Iraq).

Extraction and fractionation of phytosterol fraction of *Chenopodium murale*

1. Shade-dried coarsely powdered leaves (250g) plant will extracted with 90% ethanol (500ml) in soxhlet apparatus until complete exhaustion. The alcoholic extract was evaporated under reduced pressure at a temperature not exceeding 40 °C to give a dark green color designated as crude Fraction.
2. Crude fraction was acidified with hydrochloric acid (5%) to pH2 and partitioned (three times) with equal volume of ethyl acetate to get two layers. Aqueous acidic (F1, F2) was left and get ethyl acetate layer.
3. The ethyl acetate layer of the original alcoholic extract (crude fraction) was evaporated to dryness under reduced pressure and basified with 300ml of sodium hydroxide 5% to pH 10 and extracted with chloroform in the separator funnel to get two layers, the aqueous basic layer (F3) was left and chloroform layer was collected.
4. Chloroform layer was also separated and evaporated to dryness under reduced pressure then two types of solvents: methanol 80% and petroleum ether were added to chloroform layer to obtain phytosterol in petroleum ether layer (fraction F4, used in the study) ⁽¹⁵⁾ .

Preparation of phytosterol fraction 3% cream

Three gm of phytosterol fraction extracted from *Chenopodium murale* 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 ⁽¹⁶⁾ .

Preliminary qualitative phytochemical analysis

Chemical tests were carried out using ethanol extracts using standard procedures to identify the phytosterol fraction of *Chenopodium murale* ⁽¹⁵⁾

(I) Liebermann-Burchard test: extract (3ml) was treated with chloroform, acetic anhydride and drops of sulphuric acid were added. The formation of dark pink or red color indicates the presence of steroids.

(II)H₂SO₄ test: The development of a greenish color was considered as indication for the presence of steroids, when 2 ml of the organic extract was treated with sulphuric and acetic acids).

Qualitative and quantitative estimation of phytosterol fraction of *Chenopodium murale* using High performance liquid chromatography (HPLC) ⁽¹⁹⁾

High performance liquid chromatography was used for identification of quantitative and qualitative estimation of phytosterol fraction in the plant. The identifications will made by detection of retention time obtained at identical chromatographic conditions of steroid fraction and the standards.

Experimental condition of HPLC

- Mobile phase: ethyl acetate: water (7:30 ratio)
- Column: hyper clone ODCC C18 V-25cm ODS C18
- Column temperature: 25°C • Flow rate: 0.5ml/min
- Injection concentration 0.5mg/1ml.
- Injection volume: 20µl
- Detection wavelength: 280 nm

Treatment protocols, parameters, and animal sacrificing

The topical applications of Tacrolimus 0.1% ointment⁽¹⁷⁾ and Phytosterol Fraction of *Chenopodium murale* cream 3% (18) were applied on atopic dermatitis area of animal for 21 days once daily at 9 AM starting from the fifth day of induction.

Parameters are used to compare the results were WBC, eosinophil, serum IgE, IL4 IL13, and histopathology of AD skin lesion and compared with those of controls, and then we determine the observational severity score.

After 21 days of treatment, we took 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. Before sacrificed; blood sample collected (1ml) in EDTA tube for CBC and serum IgE, then sacrificed

by cervical dislocation; (blood sample collected by Cardiac puncture (one ml) by using (three ml syringe) in EDTA tube for CBC and serum IgE. After that Cervical dislocation for the mice was done, then atopic dermatitis skin area was cut by sharp blade (no.15). 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.

Dorsal skin samples were collected from each animal in study groups and fixed in 10% formaldehyde paraffin embedded and cut into 6 μ m sections. Deparaffinized sections were stained with ordinary hematoxylin and eosin (H&E) to determine inflammatory degree and histological changes associated with atopic dermatitis⁽²⁰⁾.

Histopathological follow-up procedures were used for the skin samples taken from each group on the 21 days of treatment. Histopathology of skin of each specimen were evaluated and scored by semi quantitative scoring systems. Histopathology included epidermal thickness, 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)⁽¹⁴⁾, the sections 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), 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⁽²¹⁾ 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 membrane; the homogenate were centrifuged for 10 minute 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.

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 gives 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⁽²²⁾.

Skin tissue homogenate of IL4 and IL13: ELISA Kit for the estimation of IL4 and IL13 was obtained from CUSABIO\China Kit was established on the base of sandwich enzyme-linked immunosorbent assay technology. Anti- IL4 and Anti- IL13 antibodies were pre-coated onto 48-well plates. And as detection antibodies, the biotin conjugated Anti- IL4 and anti- IL13 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. The concentration of IL4 and IL13 in each sample was expressed in pg/ml for comparison of results with those of controls concentration⁽²³⁾.

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⁽²⁴⁾.

Statistical analysis

Microsoft Excel 2016 and SPSS 24 were used for data entry and analysis. Numerical variables were expressed as mean \pm SD while categorical variables were expressed by frequencies and percentages, then represented by Figures and tables. All statistical comparisons were made using one -way ANOVA test. P <0.05 was considered statistically significant.

Results

High performance liquid chromatography (HPLC) for examination of phytosterol fraction of Chenopodium murale

Qualitative and quantitative estimations of active constituents of Chenopodium murale fraction was done, in which identifications was made by comprising the retention times obtained at identical chromatographic conditions of analyzed samples; the results show the presence of Beta sitosterol as a major constituent. Table 1.

Table 1. Retention time of standard and sample of Beta sitosterol of Chenopodium murale

subject	Retention Time of Stander/min	Retention Time of Sample/min	Area of Sample
Beta sitosterol	2.547	2.517	822409

The Comparison between ALL study groups (healthy control, negative control, test compound and Positive control) regarding WBC, Eosinophil, serum IgE, and skin tissue homogenate of IL-4 and IL-13, histopathology changes and scores

In comparison between the effect of topical Tacrolimus and phytosterol fraction among all study groups, the level of WBC, eosinophil, IgE, IL4 were significantly lower after tacrolimus treatment among studied groups, ($P < 0.05$) Table 5.

The effect of positive control (topical Tacrolimus) on the epidermal thickness,

Extracellular Edema, and inflammatory cells were significantly reduced, $p < 0.05$. While high

significant reduction in IL3, parakeratosis, erosion and score of observational severity was observed among test compound (*Chenopodium murale* treated group). $P < 0.001$ and $p = 0.028$ respectively.

Hyperkeratosis shows high significant reduction among both positive control and test compound (Tacrolimus and *Chenopodium murale* treated groups), $P < 0.001$. Table 2, Figure 2, 3 and 4.

Table 2 . Comparison between ALL study groups (Healthy mice, Negative control, test compound, and positive control treated groups) regarding WBC, Eosinophil, serum IgE, and skin tissue homogenate of IL-4 and IL-13, histopathological changes and score

Variables	Groups (Mean±SD)				P*
	Healthy control	Negative control	Test compound	Positive control	
WBC ($\times 10^9$ /L)	3.3 ± 2	10±2.1	7.06± 2.01	6.03± 2.02	0.01*
Eosinophil ($\times 10^9$ /L)	0.0	2.5±0.02	0.025±0.09	0.020± 2.02	0.001*
IgE(ng/ml)	15.59±8.65	26.62±5.15	16.50±6.61	16.0±6.08	0.003*
IL13 (pg/ml)	22.3±68.76	57.8±10.53	31.63±12.31	31.82±21.3	<0.001*
IL4 (pg/ml)	6.68±3.01	22.11±6.21	9.68±2.88	9.05±4.03	<0.001*
Epidermal Thickness	0.0	3.50±0.52	2.20±0.78	1.20±1.22	<0.001*
Hyperkeratosis	0.0	3.00±0.81	1.60±0.51	1.60±0.51	<0.001*
Parakeratosis	0.0	3.40±0.69	1.00±0.003	1.20±0.78	<0.001*
Erosion	0.0	1.50±0.52	0.20±0.35	0.20±0.42	<0.001*
Inflammatory Cell	0.0	2.60±0.51	1.80±0.42	1.70±0.42	<0.001*
Extracellular Edema	0.0	2.50±0.52	1.23±0.42	1.20±0.51	<0.001*
Observational Severity Score	0.0	10.00±0.81	3.50±0.97	4.50±1.08	<0.001*

*One way ANOVA test where p significant at < 0.05 and high significant at <0.001

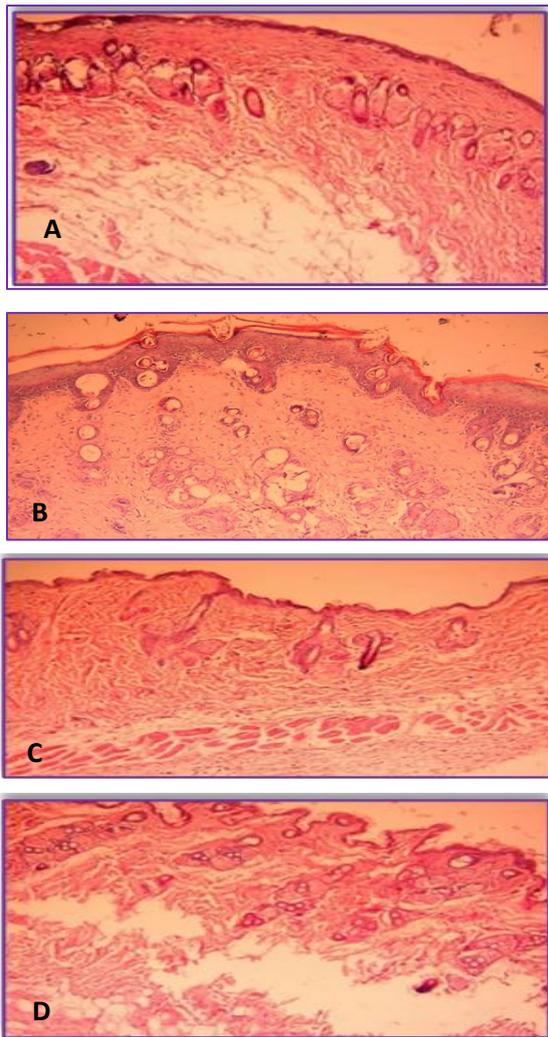


Figure 2. Histopathology changes in negative control group (B) in comparison with healthy controls (A), test compound group (C), and positive control group (D) (10x): ordinary Hematoxylin and eosin stain.

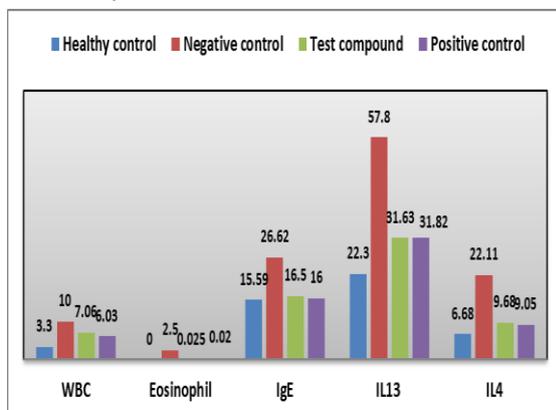


Figure 3 Comparison between negative control, healthy controls, test compound, and positive control groups regarding WBC, Eosinophil, serum IgE, and skin tissue homogenate of IL-4 and IL-13 in mice; Results are expressed as mean \pm SD by one way ANOVA test, P is significant at < 0.05 .

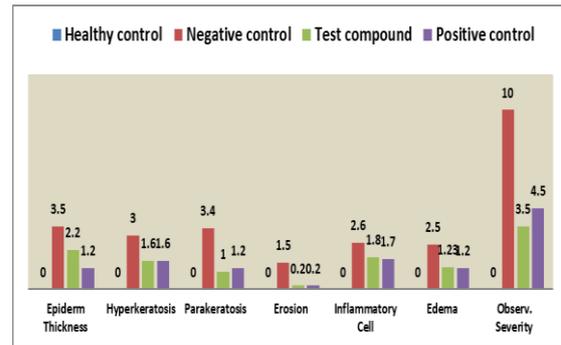


Figure 4. Comparison between negative control, healthy controls, test compound, and positive control groups regarding histological changes and observational score in mice; Results are expressed as mean \pm SD by one way ANOVA test, P is significant at < 0.05

Discussion

Atopic dermatitis (AD) is the most common chronic inflammatory and chronically relapsing skin disease. The disease leads to a significantly reduced quality of life. The pathogenesis of AD is not well understood but appears to be associated with the activation of innate immune responses, including inflammation (25, 26). Apparently, AD induced untreated group shows significant inflammation signs and significant increase in thickness and in the level of observational severity score among AD induced untreated group. Similarly; a study reported that significant increase in all types of WBC was found among AD induced untreated group (27).

Histopathology changes and observational scores after application of 3% cream topically of phytosterol fraction show a significant decrease when compared with untreated induced AD group. This indicates that the anti-atopic effect of β -Sitosterol (HPLC for examination of phytosterol fraction of *Chenopodium murale* was done, and the results show the presence of Beta sitosterol as a major constituent) is similar to the effect of tacrolimus. β -Sitosterol is attributable to the regulation of inflammatory mediator as in study concluded that the anti-inflammatory activities of β -Sitosterol could be attributed to the inhibition of inflammatory cytokine in AD-like skin lesion and reported the effect of β -Sitosterol as a therapeutic use in inflammatory skin diseases such as AD. (28) A study on animals have indicated that β -Sitosterol reduces the secretion of pro-inflammatory cytokines, as well as edema and increases anti-inflammatory cytokines (29).

Similar to the effect of phytosterol fraction of *Chenopodium murale* topical treatment, application of Tacrolimus 0.1% topically was significantly associated with reduction; as compared with non-treated induced AD group, in the levels of WBC, eosinophil, skin tissue homogenate of IL-4-

and IL-13, serum IgE, observational severity score, and histopathological changes.

A significant improvement in erythema, pruritus, sleep pattern, and quality of life was reported by 4 week tacrolimus treatment study (30). In addition to its ability in to preventing, delay, and reduce the occurrence of disease exacerbation in adult and children. ^(31, 32)

The levels of parakeratosis and observational Severity Score after phytosterol fraction of *Chenopodium murale* treatment were lower than after Tacrolimus treatment. While WBC, epidermal thickness and inflammatory cells shows more significant decrease among Tacrolimus treated group. ^(18, 28)

These results indicate that phytosterol fraction of *Chenopodium murale* that targeting IgE, IL4, and IL13 resemble the effectiveness of previously approved drug Tacrolimus ointment 1%; therefore it may be promising a good treatment for atopic dermatitis.

Conclusion

Topical application of phytosterol fraction of leafs of *Chenopodium murale* seems to be effective in treatment of atopic dermatitis through their abilities to decrease WBC, eosinophil, s. IgE, skin tissue homogenate of IL4, and IL13; as well as improving histopathology picture and reducing observational severity score. The use of phytosterol fraction of *Chenopodium murale* that target IgE, IL4, and IL13 could be promising in the treatment of atopic dermatitis.

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