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RESEARCH ARTICLE

Effect of Topical Artemisinin from *Artemisia Annua* in Comparison with Tacrolimus on Induced Atopic Dermatitis in Mice

Mohammed Fareed Hameed[1]*, Enas Jawad Kadhim[2], Ahmed Rahmah Abu-Raghif[1]

1. *Department of pharmacology and therapeutics - College of Medicine, Al-Nahrain University.*
2. *Department of pharmacognosy-College of pharmacy-Baghdad University.*

***Corresponding Authoor:** *Mohammed Fareed Hameed*

Abstract

Objective: To evaluate the effectiveness of artemisinin isolated from *Artemisia annua* terpenes fraction in atopic dermatitis mice model. Methods: This research is a randomized, prospective, placebo and controlled animal study. Thirty two male, albino mice included in this study. The mice were randomly divided into four groups (Each group eight). Group I without treatment (Healthy). Group II only the inducer Phthalic anhydride given. Groups II, III, and IV subjected to 5% phthalic anhydride solution and after three hours of phthalic anhydride application, treatment is used for group III (Tacrolimus 0.03% ointment) , and group IV (Artemisinin 175mg/kg). Artemisinin was extracted from *Artemisia annua* and isolated by preparative high pressure liquid chromatography. Measurement of serum interleukin 13, interleukin 4, and immunoglobulin E and histopathology were done. Results: When healthy group is compared to atopic dermatitis induced non-treated group, a significant increase (P value ≤ 0.05) in serum IL-13 and IL-4 were found, while high significant increase (P value ≤ 0.001) in serum IgE was recorded. Regarding Histopathology score, high significant decrease in epidermal thickness, hyperkeratosis, parakeratosis, erosion, inflammation and edema after 4 weeks of starting treatment between Tacrolimus and atopic dermatitis induced non-treated groups. When artemisinin and atopic dermatitis induced non-treated groups were compared, a significant decrease was found in serum IgE. Conclusion: *Artemisia annua* artemisinin has an effect on phthalic anhydride induced atopic dermatitis albino mice like model.

Keywords: *Atopic dermatitis, Artemisia annua, Artemisinin.*

Introduction

Atopic dermatitis (AD) characterized by acute outbreak of dry pruritic skin lesions [1]. Atopic dermatitis started during infancy and early childhood and it occurs worldwide with different prevalence according to the country and ethnic root. It is a wide spread chronic inflammatory skin disease [2]. There is an association between atopic dermatitis state and quality of life regarding body and mental health in adults [3].

It was found that the theory of Th(T helper)$_1$/Th$_2$ ratio commands the rule of pathophysiology of immune imbalance in atopic diseases with enhancement of Th$_2$ and diminishes of Th$_1$ response [4]. Th$_2$ cells can secrete IL (Interleukin)-4, IL-5, and IL- 13 and they are the cells responsible for the development of production of Immunoglobulin E (IgE). The differentiation from B cells into a plasma cells that secretes IgE occurred by IL-4 induction through enhancement of a recombinase which allows the production of IgE antibody through that the nucleus attachment of the γ domain part of the heavy chain into the junction and light part chain domain and lead to isotype switch [5].

Topical corticosteroids are almost the group of choice for treatment of atopic dermatitis and they are useful in the therapy of both children and adults [6]. Tacrolimus is effective in non-sensitive areas and sensitive area with hand eczema is the only possible exception [7]. Therefore, a safe and effective AD treatment therapy is required to establish better treatment result and fewer

side effects. *Artemisia annua* used because of its various phytochemical constituents and has many application in medicine. *Artemisia annua* has been recognized since 1970s around the entire world after the detection of the only natural phytomedicine resources for production of this plant constituent of the antimalarial lactone compound artemisinin [8].

In addition to artemisinin antimalarial properties, a lot of researchers have found that artemisinin and its structurally related compounds have many other useful pharmacological effects, like antimicrobial, cancer therapy, immunological process regulation, and anti-allergic effect in fighting both traumas induced neuronal injuries and neurodegenerative disease [9]. The objective of this study, to evaluate the effectiveness of artemisinin isolated from *Artemisia annua* terpenes fraction in atopic dermatitis mice model.

Materials and Methods

Study Design

This research is a randomized, prospective, placebo and controlled animal study. The study was done in the section of pharmacology in college of medicine Al-Nahrain University. Thirty two male Albino mice that are six weeks age included in this study. The protocols for the animal experiment used in this study were carefully reviewed for ethical and scientific care procedures and approved by Al- Nahrain University - college of medicine Committee (Approval date 4/2/2018).

The mice were randomly divided into four groups (Each group eight). Group I without treatment (Healthy). Group II only inducer, Phthalic anhydride (Prepared by dissolving phthalic anhydride in 4:1 of freshly mixed aceton and olive oil) [10] given. Groups II, III, and IV subjected to 100 microliter of 5% phthalic anhydride solution which was applied on the dorsum of the back skin at 9 A.M. three times a week for four weeks to induce a state that resemble atopic dermatitis.

After three hours of phthalic anhydride application, treatment is used for group III (Tacrolimus 0.03% ointment) [11], and group IV (Artemisinin 175mg/kg ointment) [12; 13] topically once daily at 12 P.M. for three times a week for four weeks.

Preparation of Artemisinin

The plant *Artemisia annua* is collected from north of Iraq. The plant dried and saved in AL Jadria herbal store according to the document from university of Baghdad - college of science - department of biology Approval in 12-4-2017. Five hundred grams of shad dried *Artemisia annua* leaves coarse powder were macerated in hexane for 24 hours and then dried at room temperature. The defatted plant materials were extracted with ethanol 80% in soxhlet apparatus. The ethanolic extract is evaporated using rotary evaporator at temperature not exceeding 40 Cilicia's degree.

This crude fraction was acidified with the addition of hydrochloric acid (5%) to reach pH 2 and then equal volume of ethyl acetate is added to get two separated layers. The ethyl acetate layer was evaporated to dryness using rotary evaporator under reduced pressure and then basified with 300ml of sodium hydroxide 5% to reach pH 10 and extracted with chloroform in the separator funnel to get two separated layers.

The chloroform neutral layer was separated and also evaporated to dryness then partitioned with methanol 80% and petroleum ether to get two separated layers. The methanol layer contain terpenes and steroids fractions [14]. Artemisinin is isolated and purified by using preparative high pressure liquid chromatography**.**

The purification process mostly consists of different consecutive purification in order to gate pure active compound for structure identification because the original crude extract is an extremely complex mixture. Requirements for the purification and isolation system of good quantity and large number of herbal compounds were required, which was depending mostly on preparative high performance liquid chromatography [15].

Measurement of Serum Interleukin 13, Interleukin 4, and Immunoglobulin E:

Serum interleukin 13, interleukin 4, and immunoglobulin E measured quantitatively by the enzyme-linked immunosorbent assay (Using mice serum IL-13 kit, catalog number: YHB0790Mo, SHANGHAI YEHUA Biological Technology-China; mice serum IL-4 kit, catalog number: M4000B, R&D Systems-

USA; and mice serum IgE kit, catalog number: CSB-E07983m, Cusabio-China). After incubating the tested serum in an antigen-coated polystyrene plat or tube, enzyme specifically labeled anti-immunoglobulin is then added and this enzyme then remaining in the plate or tube after washing gives a measure to the quantity of specifically related antibody in the serum [16].

Histopathology Procedure

All mice weighted before and after the study. At the end of the study the mice are anaesthetized by chloroform to effective dose by inhalation, blood sample collected and sacrificed by cervical dislocation on the 28th day after starting treatment. Skin tissue is then collected from target sites and stored in buffered formaldehyde [17]. Semi quantitative scoring systems for the evaluation of mouse model histopathology include epidermal thicking, parakeratosis, hyperkeratosis, inflammation, ulcer, and edema (Each given number range 0-4) [18] has been done.

Statistical Analysis

Statistical analysis was done by analyzing data using computer facilities of statistical package for the social sciences version-25 and tests of mean, standard deviation, and independent t-test were done for analysis of variance used to test the significance of difference and was considered statistically significant if the probability value (P value) was less or equal to 0.05 and highly significant if the probability value (P value) was less or equal to 0.001 [19].

Results

Healthy serum IL-13, IL-4 and IgE mean± SD were 36.56±1.76, 8.02±4.17, and 2.26±3.06 respectively. When healthy group is compared to atopic dermatitis induced non-treated group, a significant increase (P value ≤ 0.05) in serum IL-13 and IL-4 were found, while high significant increase (P value ≤ 0.001) in serum IgE was recorded.

Also, high significant increase in histopathology score including epidermal thickness, hyperkeratosis, parakeratosis, erosion, inflammation and edema were shown, while no significant difference in Ulcer was recorded. Table (1). Table (2) indicates after 4 weeks of starting treatments, no significant differences in Serum IL-13, a significant decrease in serum IL-4 and high significant decrease in serum IgE were found between tacrolimus and atopic dermatitis induced non-treated groups.

Regarding histopathology score, although no significant difference found in ulcer parameter, high significant decrease in epidermal thickness, hyperkeratosis, parakeratosis, erosion, inflammation and edema after 4 weeks of starting treatment between tacrolimus and atopic dermatitis induced non-treated groups.

When artemisinin and atopic dermatitis induced non-treated groups were compared, no significant difference was found in serum analysis of IL-13 and IL-4 and a significant decrease was found in serum IgE. In addition to that, for histopathology score, although no significant difference found in ulcer parameter, a significant decrease in edema and high significant decrease in epidermal thickness, hyperkeratosis, parakeratosis, erosion and inflammation were found Table (3).

Table 1: Comparison between healthy group and atopic dermatitis induced non-treated group

parameter	Healthy group mean± SD	Atopic dermatitis group mean± SD	p- value
Serum IL-13 (pg/mL)	36.56±1.76	66.35±28.2	0.004*
Serum IL-4 (pg/mL)	8.02±4.17	22.81±12.31	0.002*
Serum IgE (ng/ml)	2.26±3.06	22.88±13.95	<0.001**
Epidermal thickness	0.0±0.0	3.0±0.0	<0.001**
Hyperkeratosis	0.0±0.0	3.0±0.0	<0.001**
Parakeratosis	0.0±0.0	3.0±0.0	<0.001**
Erosion	0.0±0.0	1.0±0.0	<0.001**
Inflammation	0.0±0.0	2.0±0.0	<0.001**
Edema	0.0±0.0	2.11±1.05	<0.001**
Ulcer	0.0±0.0	0.0±0.0	1.0

* Denote significant difference at P value ≤ 0.05

** Denote high significant difference at P value ≤ 0.001

Table 2: Comparison between atopic dermatitis induced non-treated group and tacrolimus group:

parameter	Atopic dermatitis group mean± SD	Tacrolimus group mean± SD	p- value
Serum IL-13 (pg/mL)	66.35±28.2	63.74±15.41	0.82
Serum IL-4 (pg/mL)	22.81±12.31	10.55±1.89	0.014*
Serum IgE (ng/ml)	22.88±13.95	2.67±4.78	0.001**
Epidermal thickness	3.0±0.0	1.0±0.0	<0.001**
Hyperkeratosis	3.0±0.0	0.0±0.0	<0.001**
Parakeratosis	3.0±0.0	0.0±0.0	<0.001**
Erosion	1.0±0.0	0.0±0.0	<0.001**
Inflammation	2.0±0.0	1.0±0.0	<0.001**
Edema	2.11±1.05	1.0±0.0	0.001**
Ulcer	0.0±0.0	0.0±0.0	1.0

* Denote significant difference at P value ≤ 0.05
** Denote high significant difference at P value ≤ 0.001

Table 3: Comparison between atopic dermatitis induced non-treated group and artemisinin group:

parameter	Atopic dermatitis group mean±SD	Artemisinin group mean±SD	p- value
Serum IL-13 (pg/mL)	66.35±28.2	48.07±6.41	0.094
Serum IL-4 (pg/mL)	22.81±12.31	13.66±3.32	0.06
Serum IgE (ng/ml)	22.88±13.95	3.45±3.89	0.002*
Epidermal thickness	3.0±0.0	2.13±0.35	<0.001**
Hyperkeratosis	3.0±0.0	2.0±0.76	0.001**
Parakeratosis	3.0±0.0	0.0±0.0	<0.001**
Erosion	1.0±0.0	0.0±0.0	<0.001**
Inflammation	2.0±0.0	1.0±0.0	<0.001**
Edema	2.11±1.05	0.88±0.35	0.007*
Ulcer	0.0±0.0	0.0±0.0	1.0

* Denote significant difference at P value ≤ 0.05
** Denote high significant difference at P value ≤ 0.001

Discussion

Significant increase in serum IL-13 was shown in atopic dermatitis non-treated group and this is identical with the study that stated the signaling pathway that initiated by binding of the T cell to receptor form a complex leading to increased IL-13 synthesis in patients with AD [20]. This study also showed significant increase in serum IL-4 of an AD induced non treated group and it is the same result by which IL-4 synthesis by Th_2 cells which was much higher in the non-treated group than the normal healthy group [21]. Moreover, high significant increase in serum IgE was recorded in the current study.

This result was comparable with a study showed that repeated skin application of phthalic anhydride solution lead to a significant increase in serum IgE levels in the induced non-treated group [22]. The hyper-production of IgE is one of the allergic hypersensitivity features and it was indicator of the degree of the allergic immune responses in the AD developments [23].

The present study showed also high significant increase in histopathology score including epidermal thickness, hyperkeratosis, parakeratosis, erosion, inflammation and edema but not ulcer. A study in 2017 showed increased epidermal thickness and the infiltration of mast cells and eosinophils to the skin [24]. Wang found that focal-parakeratosis and hyperkeratosis were one of the histological finding in dermis of mice AD skin tissues [25]. Haematoxylin and eosin staining of the dorsal part of skin sections showed intracellular edema, hypertrophy, and inflammatory cells infiltration into the upper part of dermis in AD mice [26].

A significant decrease in serum IL-4 was found in tacrolimus treated group. The synthesis of Th_1 cytokines (Interferon-gamma and IL-2) and Th_2 cytokines (IL-4 and IL-5) is strongly inhibited with tacrolimus [27].

A study quantified the picture of inflammatory infiltration of cell in the skin lesion pre-treatment and post-treatment with topical tacrolimus compare to topical hydrocortisone. The number of infiltrated skin CD3 +, CD4 +, and CD8 + lymphocyte cells, neutrophils, and eosinophils was significantly suppressed on uses of topical tacrolimus when compared hydrocortisone.

In addition to that, topical skin tacrolimus significantly decrease the infiltrated cells number that cause express IL-4, IL-5, IL-12 IL-13, CCR3, IFN-gamma, and CXCR4, which was found more obvious with tacrolimus from hydrocortisone [28].

High significant decrease in serum IgE was found in this study in tacrolimus treatment group which is similar to the result of a study that showed tacrolimus significantly suppressed the increased serum IgE concentration [29]. Tacrolimus cause immunosuppression through decreasing responses of T lymphocytes to foreign allergic antigens in addition to suppressing IL-2 cytokine transcription which is the main pathway.

It controls transcription of several genes that code for many inflammatory mediators like IL-2, tissue necrosis factor-alpha, granulocyte-macrophage colony-stimulating factor, IFN-gamma as well as other interleukins which are required for immune responses development. Tacrolimus also suppress histamine release from mast cells [30]. Regarding histopathology score, high significant decrease in epidermal thickness, hyperkeratosis, parakeratosis, erosion, inflammation and edema was found in tacrolimus treated group when compared with atopic dermatitis induced non-treated group.

Topical tacrolimus clearly and rapidly decrease dermatitis scores, and the suppression effects become throughout the study permanently. The efficacy of tacrolimus ointments on dermatitis scores was also attach with the significant amelioration of histological parameters like inflammatory cells infiltration and epidermal thickening [31]. The atopic disorders diseases, which are atopic dermatitis, bronchial asthma and allergic rhinitis share a common disease pathogenesis which are mediated by IgE [32].

A significant decrease was found in serum IgE after 4 weeks of starting treatment between artemisinin and atopic dermatitis induced non-treated groups similar to a study where IgE and histamine concentration were extremely decrease less than the non-treated allergic rhinitis a model of mouse [33]. Atopy can be defined as a familial or personal tendency to synthesis IgE antibodies and sensitization due to environmental triggers [34].

For histopathology score, significant decrease in edema and highly significant decrease in epidermal thickness, hyperkeratosis, parakeratosis, erosion and inflammation is seen in artemisinin treated group when compared with atopic dermatitis induced non-treated group. A report for artemisinin derivative possess anti-allergic effect by suppressing IgE-induced degranulation of mast cell, giving an evidence for artemisinin derivative for the treatment of mast cell mediated allergic disease or allergic asthma [35].

Conclusion

The data suggest that *Artemisia annua* artemisinin reduces the atopic dermatitis response to phthalic anhydride sensitized albino mice via probably via the inhibition of IgE-mediated mast cell degranulation.

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