Topical Vardenafil in Comparison with Tacrolimus on Induced Atopic Dermatitis in Mice

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Abstract

Background: Atopic dermatitis is a chronic inflammatory skin disease that is characterized by recurrent pruritus, dry skin, and eczematous rash, and is extremely difficult to treat. The main therapy for the management of atopic dermatitis includes treatment with glucocorticoids and calcineurin inhibitors, problems with corticosteroid and calcineurin inhibitors in long-term use can cause side effects; therefore, a safe and effective atopic dermatitis treatment therapy is needed to establish better outcome with the least side effect.

Aim of the study: To evaluate the possible therapeutic effect of topical vardenafil in induced atopic dermatitis in mice.

Methods: The study included fifty Albino male mice of six weeks' age. The mice were randomly divided into five groups (Each group contained 10 mice). A control group without treatment (apparently healthy), and five groups (non- treated induced, vehicle, tacrolimus, and vardenafil treated group) were topically treated once daily with 1-Chloro-2,4- dinitrobenzene solution 1% and 0.2% on the dorsum of the back skin to induce atopic dermatitis. The animals were housed in a private and good ventilated isolated place; with a room temperature of 20-24°C and kept light for 12 hours. Before starting the study, the animals were left for seven days to acclimatize to the animal room conditions and allowed free access to water and Ad libitum feeding. three groups of 10 induced atopic dermatitis mice that treated with (vehicle, 1% tacrolimus, and 1% Vardenafil ointment) topically once daily at 10 AM for 3 weeks, for assessing the effect of different treatments, the flowing parameter will be evaluated, Blood picture (WBC, Eosinophil, neutrophil), histopathological score (hyperkeratosis, parakeratosis, erosion, edema, epidermal thickness, and inflammation), immunohistochemistry (IL4, IL13) and Observational severity score.

Results: Levels of WBC, Eosinophil, neutrophil, immunohistochemistry of IL-13 and IL-4, and histopathological scores (hyperkeratosis, parakeratosis, erosion, epidermal thickness, edema, and inflammation) were significantly increased among induced non treated AD group in comparison with the apparently healthy group. In comparison between the effect of the vehicle-treated group with induced non-treated group show no effect on all parameters except (mild erosion and extracellular edema) while In comparison between the effect of Tacrolimus1% ointment treated group with an induced non-treated group in relation to different these parameters show a reduction in this parameter and the most obvious decrease in the level of WBC in comparison with another treated group, the Vardenafil 1% ointment treated group in comparison with the induced non-treated group in relation to different these parameter show significant reduction in WBC, neutrophil, eosinophile, IHC (IL13, IL4), histopathological score and observational severity score. But the vardenafil treated group has an obvious ability to decrease the level of epidermal thickness, erosion, and parakeratosis Furthermore, in comparison between the effect of Tacrolimus, and Vardenafil treatment on the studied variables regarding histopathological scores; Hyperkeratosis and inflammation were significantly lower after Felodipine and Vardenafil treatment among studied groups.

Conclusion: According to the results obtained from this study, all studied groups (Tacrolimus and Vardenafil treated group) shows Obvious therapeutic effect in a model of atopic dermatitis in mice. They show significant improvement in the histopathological picture in comparison with the induced non-treated group. Both felodipine and vardenafil show anti-inflammatory effects (decrease IL4, IL13), they also decrease WBC and neutrophiles.

Keywords: Topical Vardenafil, Comparison, Tacrolimus, Induced Atopic, Dermatitis, Mice.

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ATOPIC DERMATITIS

1.1.1 Definition

Atopic dermatitis (AD) is a common chronic recurrent inflammatory skin disease field (Leung *et al.*, 2004). AD started during infancy and early childhood and it occurs worldwide with different prevalence according to the country and ethnic root it is a widespread, chronic inflammatory skin disease (Rothenbacher and Brenner, 2003). As the understanding of the association between atopy and allergic immune reaction increased, (atopic dermatitis) was used as the accepted name. Atopic eczema term has become more popular nowadays field (Spiewak, 2008).

There is an association between AD state and quality of life regarding the body and mental health in adults (Leung *et al.*, 2004).

1.1.2 Diagnostic Criteria

The diagnostic criteria for AD might have a wide resumption, not only to pediatricians and dermatologists, but also to immunologists, allergists, and geneticists, who require a valid and simple definition (Karlinger *et al.*, 2000).

The proposed fashion of the diagnostic criteria for AD shows suggested diagnostic guidelines for AD that must have an itchy skin status (or parental note of rubbing or scratching in a child) and three or more of the following:

1. A history includes skin rash for example elbows folds, behind the knees, around the neck (Including children's cheeks under 10), or in front of ankles.
2. A history of hay fever or asthma in a person (Or a previous history note of a first-degree relative in children less than four years suffer from AD).
3. A previous history of total dry skin in the previous year.
4. Noticed flexural dermatitis (or dermatitis involving the forehead/cheeks and limbs in children less than four years).
5. The onset is under the age of two (This is not used if the child is less than four years). These diagnostic guidelines are used for children, adults, and non-white ethnic subjects suffering from AD(Williams *et al.*, 1994).

1.1.3 Epidemiology

The Prevalence of atopic dermatitis in Iraq patients was 7.5%. Atopic dermatitis syndrome males represent 43.6%, while females represent 56.4%(Al-Hammamy, 2013).

Atopic dermatitis affects about 10% of adults and 20% of children in developed countries (Tsakok *et al.*, 2019).

The prevalence of eczema symptoms had been studied in the largest study in children and adolescents, where the prevalence of active eczema symptoms through the age 6 to 7 years old was different from area to an area like in Jodhpur in India about 0.9% to about 22.5% in Quito-Ecuador, while for the age range 13 to 14 years, the prevalence of symptoms differ from 0.2% in Tibet in China and reaching 24.6% in Barranquilla in Colombia. The proportional percentage of boys included varied through centers ranging from 38.2% reaching to 66.1%, and the proportional percentage of girls differ from 33.9% reaching to 61.8%. Boys established a lower percentage of prevalence of active symptoms of eczema (7.7% for boys and 8.2% for girls; $P < 0.001$). A little with no difference was found between girls and boys for severe eczema (1.0% for males and 1.1% for females) while a slightly higher non-significant prevalence was recorded for boys in lifetime dermatitis (14.4% for males, 14.1% for females) (Odhiambo *et al.*, 2009). Although the international study of asthma and allergies in childhood (ISAAC) and other epidemiologic research of AD almost did not include adults, A research of 27157 adults (Ages from18 to 85 years old) from a US National Health Interview Survey in 2010 conclude that the prevalence of eczema, dermatitis, or any red, inflamed rash of skin in the last year of the study was 10.2% (Silverberg, 2015).

1.1.4 Pathophysiology

There were several etiological factors of AD, some are called multifactorial like a combination of environmental inducers, genetic causes, and/or exposures (Dinis-Oliveira *et al.*, 2006). The clinical picture phenotypes of AD have been divided into intrinsic and extrinsic types, similar to the divisions of asthma. The intrinsic or what is called non-allergic AD is distinguished by normal total immunoglobulin E (IgE) concentration with no specific IgE. Intrinsic AD represents about 20% of all AD cases and is predominantly occurred in female patients. Furthermore, it is specifically had a relatively milder severity and late-onset. Also, the skin barrier is not troubled, in intrinsic type. While the extrinsic type is characterized by mostly high serum IgE concentration and represents 80% of all AD cases (Tokura, 2010).

MATERIALS AND METHODS

Induction of atopic dermatitis in mice:

- Mice had been sensitized with 1-Chloro-2,4-dinitrobenzene (DNCB)
- Preparation DNCB 1% and 2%: Dissolve 100 mg DNCB powder in 20 mL of acetone/olive oil (4:1; v/v) solution (2%). Half dilution makes the solution 1%.
- Groups II, III, and IV, V had subjected to shaving the hair off from the dorsal skin region with a fine electric shaver. In order to sensitize the skin and for the induction of atopic dermatitis, 100 µL of 1% DNCB in 4:1 (v/v) acetone/olive oil solution was topically applied once daily to the exposed skin for two days. Apply 120 µL of 2% DNCB on day 3. After the visual confirmation of parameters for skin sensitization, mice had been treated with test samples.

Parameters of the study

1. Complete blood count : WBC, neutrophil, and eosinophil count in blood
2. Immunohistochemistry methods: It involves the process of selectively identifying antigens (proteins) in cells of a tissue section by exploiting the principle of antibodies binding specifically to antigens in biological tissues.[21] : iL-4 and iL-13 were measured in the atopic dermatitis skin lesion.
3. Evaluation of dermatitis severity: The severity of dermatitis on the dorsal regions was evaluated. The development of erythema/hemorrhage, scarring/dryness, edema, and excoriation/erosion was scored as: 0 (none), 1(mild),2(moderate), or 3 (severe). Clinical skin score was defined as the sum of the individual scores, ranging from 0 to 12.
4. Histopathology evaluation: All mice were weighted, anesthetized by to effective dose of (ether) inhalation, and Euthanize by cervical dislocation after treatment

and then Semiquantitative scoring systems for the evaluation of mouse model histopathology include: Epidermal hypertrophy, hyperkeratosis, parakeratosis, erosion, inflammation, edema, ulcer (each 0–4) had been examined by a pathologist.

Immunohistochemistry (IHC)

Immunohistochemistry offers the benefit of direct identification of the state of cells in the specifically affected tissue (Bertevello et al., 2005). To determine cytokines that present in the lesion of mice skin, an IHC technique was initially standardized at the immunohistochemistry laboratory of the department of Microbiology with the aid of the consultation Center in the Pathology department - College of Medicine /Al Nahrain University.

STATISTICAL ANALYSIS

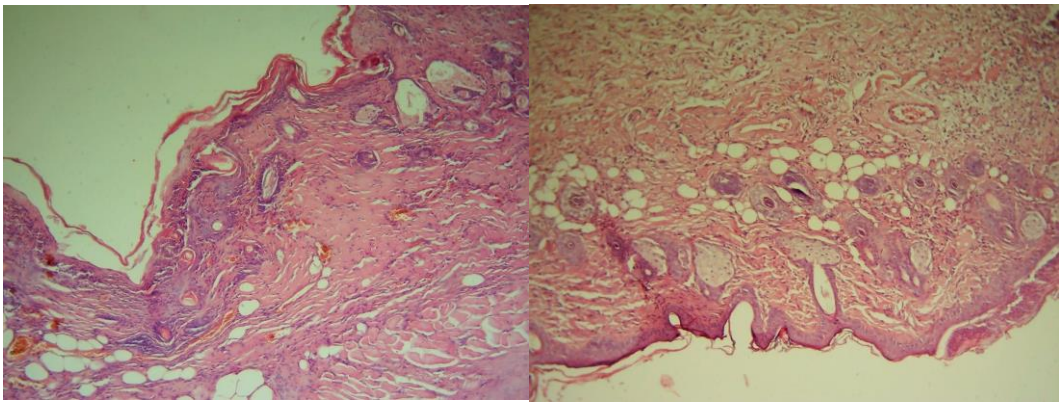
Statistical analysis was done by analyzing data using computer facilities of SPSS-25 and tests of mean, standard deviation, and independent t-test were done for analysis of variance used to test the significance of difference and consider statistically significant if the probability value (P value) less or equal to 0.05 and highly significant if the probability value (P value) less or 2. Management and treatment of atopic equal to 0.001.

RESULT

Comparison between non-treated atopic dermatitis induced group and Vardinafil treated group regarding histopathological score

		Groups				P* value
		Induced Non-treated		Vardinafil group		
		N	%	N	%	
Epidermal thickness	No abnormality	0	0.0%	10	100.0%	**<0.001**
	Slight (+1)	0	0.0%	0	0.0%	
	Mild (+2)	0	0.0%	0	0.0%	
	Moderate to severe (+3)	10	100.0%	0	0.0%	
Hyperkeratosis	No abnormality	0	0.0%	10	100.0%	**<0.001**
	Slight (+1)	0	0.0%	0	0.0%	
	Mild (+2)	0	0.0%	0	0.0%	
	Moderate to severe (+3)	10	100.0%	0	0.0%	
Parakeratosis	No abnormality	0	0.0%	10	100.0%	**<0.001**
	Slight (+1)	0	0.0%	0	0.0%	
	Mild (+2)	0	0.0%	0	0.0%	
	Moderate to severe (+3)	10	100.0%	0	0.0%	
Erosion	No abnormality	0	0.0%	10	100.0%	**<0.001**
	Slight (+1)	0	0.0%	0	0.0%	
	Mild (+2)	0	0.0%	0	0.0%	
	Moderate to severe (+3)	10	100.0%	0	0.0%	
Inflammation	No abnormality	0	0.0%	10	100.0%	**<0.001**
	Slight (+1)	0	0.0%	0	0.0%	
	Mild (+2)	0	0.0%	0	0.0%	
	Moderate to severe (+3)	10	100.0%	0	0.0%	
Edema	No abnormality	0	0.0%	10	100.0%	**<0.001**
	Slight (+1)	0	0.0%	0	0.0%	
	Mild (+2)	0	0.0%	0	0.0%	
	Moderate to severe (+3)	10	100.0%	0	0.0%	

***Chi^2 test, P significant at ≤ 0.05**



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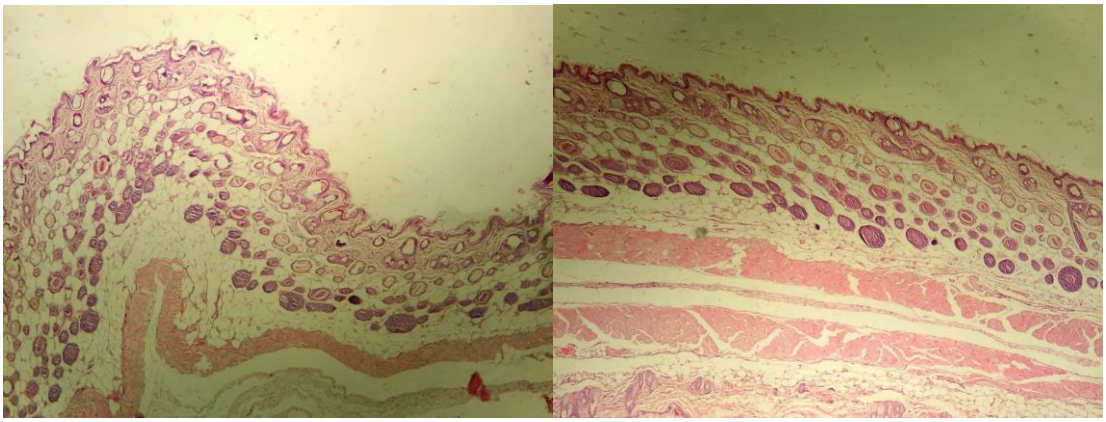

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Figure 10: Histopathology changes in topically induced AD group (C) in comparison with Vardenafil (D) (10x): ordinary Hematoxylin and eosin stain.

3. Comparison between studied groups (Tacrolimus treated group, and Vardenafil treated group) in relation to different parameters

In comparison between the effect of Tacrolimus, and Vardenafil treatment on the studied variables regarding WBC, Eosinophil, neutrophil, and IHC of IL-4 and IL-13; the level of WBC was significantly lower after tacrolimus treatment among the studied groups (P=0.007). While the level of neutrophil was significantly lower after vardenafil treatment among the studied groups, P<0.001. Tables 16 & 17. Figures 11, 12 & 13.

No significant difference was found among all groups according to the eosinophil level and IHC of IL4- and IL-13, (P=0.06 and P= constant) respectively. Tables 16 & 17. Figures 11, 12 & 13.

DISCUSSION

Atopic dermatitis (AD) is a chronic inflammatory & persistent skin disease characterized by itching and multiple eczematous lesions. The prevalence of inflammatory and allergic skin diseases in developing countries is estimated to be very high. Atopic dermatitis and allergic form of dermatitis are still managed by using calcineurin receptor inhibitors and steroids as the main drug treatments. However, in spite of being a serious health problem, the common different clinical treatments show limited outcomes and are usually accompanied by side effects (chun.2020). Although many biological agents have been developed recently with very good therapeutic effects, they are of high price and this limits their accessibility. Therefore, there is a vital need to develop effective reasonably priced, and original therapies to treat this condition. This study is to evaluate the effect of topical Felodipine and Vardenafil in the treatment of atopic dermatitis in mice model in relation to different parameters including blood picture (WBC, neutrophil, eosinophil), immunohistochemistry inflammatory markers (iL-4 and iL13), and a histopathological score of skin (epidermal thickness, hyperkeratosis, parakeratosis, erosion, Inflammation, and extracellular edema).

Comparison between non-treated atopic dermatitis (induced group) and Vardenafil treated group

Levels of WBC, Neutrophil, Eosinophil, IHC of IL4 and IL13 were affected by the treatment with Vardenafil which appears significant decreased from those of the non-treated induced AD group, but the histopathological scores (epidermal

thickness, hyperkeratosis, parakeratosis, inflammation, and extracellular edema) was had mainly a significant reduction after treatment with Vardenafil.

The postulated decrease in the counts of total WBCs, eosinophils, neutrophils, and IL13&4 may be considered as a possible mechanism of an anti-inflammatory effect of vardenafil which is also considered as a side effect for it, which can be used in future experiments as a promising topical agent for the treatment of AD. These findings are inconsistent with a previous review of literature by (Halim and Ogbeide, 2002) that considers this potential activity as a side effect. Additionally, vardenafil has a significant effect on eosinophil function. This inhibitory effect of vardenafil is similar to dapsone's potential effect on eosinophil peroxidase as a previous explanation of the therapeutic efficacy in eosinophil-mediated diseases showed that the dapsone, like vardenafil, inhibitory effect reduces the effect of eosinophil peroxidase on mast cells which cause decreasing the release of the inflammatory mediators, hence the inhibitory effect might also be due to the inhibition of lipoxygenase and cytokines production (Smith et al. 2008).

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