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A comparative study to examine the effects of topical Vinpocetine and Tacrolimus on induced atopic dermatitis in mice.

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Abstract

Atopic dermatitis (AD) is a persistent and recurring inflammatory skin disorder distinguished by parched skin and severe itching. The pathogenesis of AD involves increased production of specific cytokines, such as Interlukin-4 (IL-4) and Interlukin-13 (IL-13), which are associated with the T helper 2 pathway. However, prolonged use of the current glucocorticoids and calcineurin inhibitors medications can lead to adverse effects. **Aim of the study:** To assess the efficiency of topical Vinpocetine and Tacrolimus in treating an induced atopic dermatitis mice model. **Methods:** Five sets of fifty male Albino mice, were randomly selected. One-chloro-2,4-dinitrobenzene (DNCB) was applied to the dorsal (back) skin of forty mice to cause atopic dermatitis. Ten control healthy mice were placed in Group I, ten mice with atopic dermatitis were placed in Group II; neither group received any treatment. Group III consists of 10 mice that received tacrolimus 0.1% ointment, group IV was administered a topical ointment comprising 5% Vinpocetine and lastly, group V underwent a vehicle ointment. Severity was assessed visually, IL-4 and IL-13 were measured by immunohistochemistry, in addition to general histopathological evaluation as well as examining WBCs. **Results:** The groups treated with Vinpocetine 5%, and Tacrolimus all had significantly lower levels of WBC counts, decreased IL-4 and IL-13 staining according to immunohistochemistry and lower histopathological scores. Vinpocetine 5% and tacrolimus treatment also showed a statistically significant reduction in hyperkeratosis and inflammation among the studied groups ($P<0.001$). **Conclusions:** Topical Vinpocetine 5% ointment, and tacrolimus 0.1% were effective in the treatment of induced AD mouse model through the improvement of histopathological changes and their ability to decrease IL-4 and IL-13, Vinpocetine were effective in the treatment of induced AD.

مقارنة بين الفينبوسيتين الموضعي مع التاكروليماس في التهاب الجلد التأتبي المستحدث في الفئران

الحسن هيثم حباس احمد رحمه ابو رغيف

الخلاصة

التهاب الجلد التأتبي او الاكزيما هومرض جلدي مزمن متكرر يتميز بالجفاف (زيادة فقدان الماء من خلال الجلد) و يسبب الحكة وطفح جلدي. يتميز التهاب الجلد التأتبي بارتفاع إنتاج السيتوكينات المرتبطة بمسار خلايا تي المساعده صنف 2مع الانترلوكين 4 والانترلوكين13 والتي تعد من العوامل الرئيسية في هذا المرض. من المقبول عمومًا أن الجلوكوكورتيكويدات ومثبطات الكالسينيورين هي الخطوط الأولى لعلاج التهاب الجلد التأتبي. ومع ذلك، قد تكون هناك آثار جانبية من استخدام مثبطات الكالسينيورين أو الكورتيكوستيرويدات لفترة طويلة من الزمن. الهدف من الدراسة: معرفه وتقييم مدى الفعاليه الدوائيه للفنبوستين والتاكروليموس لألتهاب الجلد التأتبي في نموذج فأر أصيب بمرض التهاب الجلد التأتبي. الطريقة: تستخدم هذه الدراسة تصميمًا عشوائيًا يتم التحكم فيه باستخدام الحيوانات. تم أخذ 50 فأر ذكر من ذكور البينو وتم تقسيمها بشكل عشوائي إلى خمس مجموعات. عولج جلد ظهر أربعون فأرًا بـ محلول ثنائي نترو كلوروبنزين للحث على التهاب الجلد التأتبي، ثم تم تقسيم الفئران بشكل عشوائي إلى خمس مجموعات. كان هناك 10 فئران تحكم (تبدو صحية) في المجموعة الأولى و 10 فئران مصابة بالتهاب الجلد التأتبي في المجموعة الثانية، المجموعة الثالثة تداوت بالتاكروليموس 0.1٪ موضعيًا ،تلقت المجموعة الرابعة مرهمًا موضعيًا يحتوي على فينبوستين 5% بينما تلقت المجموعة الخامسة المرهم الأساسي فقط بدون مادة علاجيه. تم فحص الأنسجة ، والكيمياء المناعية لإنترلوكين 4 وإنترلوكين 13، وسجلت درجة شدة الاصابة، وقياس مستويات كريات الدم البيضاء في دم الفئران. النتائج: في المقارنات بين المجموعه الغير معالجة المصابة بالتهاب الجلد التأتبي و المجموعة المعالجة الفينبوستين 5 % أو المجموعه المعالجة بالتاكروليماس0.1% و يظهر انخفاضا كبيرا في عدد خلايا الدم البيضاء ،وانخفاض الانترلوكين 13وانترلوكين 4 لتحليل الكيمياء النسيجية المناعية و درجة الانسجة المرضية. بعد تطبيق الفينبوستين يظهر انخفاض معنوي في جميع المعلمات بقيمة معنوية اقل من 0.05 .أهم انخفاض هو في مستوى الالتهاب وفرط التقرن في الدرجات النسيجية المرضية ودرجة الشدة المسجلة التي لوحظت بين المجموعة المعالجة بالتاكروليماس والفينبوستين حيث ان القيمة المعنوية اصغر من 0.001 . الاستنتاجات: وجد ان التطبيق الموضعي لمرهم الفينبوستين 5% وتاكروليموس 1٪ يبدو أنه فعال في علاج نموذج الفئران المستحث بمرض التهاب الجلد التأتبي .

Introduction

Atopic dermatitis (AD) is a pruritic, chronic relapsing and remitting inflammatory skin disease that usually commences in early infancy and childhood, it's also termed (atopic eczema); is the most common skin disorder characterized by an acute outbreak of dry pruritic skin lesions. AD is characterized by eczematous lesions, lichenification, pruritus, susceptibility to infection and xerosis (dry skin) [1, 2]. Atopic dermatitis could be connected to other atopic conditions, including acute allergic responses to foods, asthma, urticaria, and allergic rhinitis that are induced as a result of excessive immunoglobulin E (IgE) presence and filaggrin protein under expression where atopic diseases that initiate typically atopic dermatitis during infancy can lead to the development of allergic rhinitis and/or asthma in later stages. [3] Atopic dermatitis is childhood-related disease that arises in 85% of patients at the age that is below 5 years old, however, atopic dermatitis may resolve by adolescence.[4] In developed nations, AD is among the most prevalent skin conditions, impacting approximately 20% of children and 1% to 3% of adults. Furthermore, in industrialized countries, the incidence of AD has risen by 2 to 3 times.[5] The condition is slightly more prevalent in males than in females. Familial factor plays a major role and the emergence of a topic dermatitis and children where 60% of adults with a topic dermatitis have children with a topic dermatitis as well and the incidence is higher when both parents have atopy (81%) [6] [2]. Environmental risk factors also have a crucial impact on AD occurrence these factors encompassing climate, diet, industrialized lifestyle (urbanization), breastfeeding, environmental air pollutants, obesity and physical exercise or tobacco smoking have been

implicated to be as risk factors for AD. [7] The exact cause of atopic dermatitis remains incompletely understood. However, its development involves a multifactorial process with interconnected immunological mechanisms. The onset of atopic dermatitis is multifactorial and can be attributed to several key factors. Firstly, barrier dysfunction plays a significant role, as compromised skin barriers can allow external irritants to penetrate more easily. Secondly, alterations in cell-mediated immune reactions within the skin. Thirdly, the involvement of IgE-mediated hypersensitivity reactions, with immune responses driven by allergen-specific IgE antibodies. Moreover, environmental factors, such as exposure to allergens, pollutants. Genetic predisposition is another critical factor, as individuals with a family history of the condition are more susceptible. Finally, the diverse array of immune cell types, including T cells, B cells. [8]. Despite recent progress in comprehending the genetics of atopic dermatitis, its pathophysiology remains inadequately characterized. There is a need for further investigation to uncover the underlying mechanisms of atopic dermatitis and to devise more efficient treatment approaches.[9]. Vinpocetine is a semi-synthetic alkaloid derived from Periwinkle (*Vinca minor*) leaves. Vinpocetine is a derivative of apovincamine, and it was developed around 1978 under the trade name of Cavinton. Vinpocetine has been used for the treatment of cerebrovascular and cognitive disorders since the 1970s. Vinpocetine demonstrated anti-inflammatory activity in vascular smooth muscle cells, monocytes, endothelial cells, neutrophils, epithelial cells, macrophages, brain microglial cells and dendritic cells by direct inhibition of IκB kinase thus withholding phosphorylation of IκB protein and consequently the expression of inflammatory pathways dependent on nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) is inhibited.[10] [11] Vinpocetine improves brain perfusion by acting as a vasodilator agent as well as vinpocetine promotes cerebral metabolism via raising glucose and oxygen uptake and stimulating neuronal ATP production. [12] Tacrolimus is a very potent anti-T-lymphocyte, macrolide, and immunosuppressant medicine produced from the fungus *Streptomyces tsukubaensis*. The topical application of tacrolimus at a concentration of 0.03% to 0.1% has been reported to possess effectiveness therapeutically in common inflammatory skin diseases such as psoriasis and AD, among pediatric (middle childhood [2-6 years] and school aged [7-15 years] children) and adult patients [4-6]. A lower concentration (0.03%) in the pediatric age cluster is recommended and is contraindicated in the usage below two years of age.[13] There are several issues and challenges associated with studying vinpocetine and comparing it to the standard treatment in the context of atopic dermatitis. The utilization of vinpocetine for atopic dermatitis faces several significant challenges. First and foremost, the scarcity of well-designed clinical trials dedicated to vinpocetine's efficacy in managing this condition makes it difficult to establish its safety and effectiveness definitively. Safety concerns surrounding vinpocetine use in this context also require comprehensive investigation to assess potential side effects and interactions with other medications. Furthermore, the limited financial support and industry interest in vinpocetine research could impede progress in conducting large-scale, controlled trials necessary for a better understanding of its role in managing atopic dermatitis.[14] This study was aimed to investigate the efficiency of topical Vinpocetine and Tacrolimus in treating an induced atopic dermatitis mice model.

Methods and material

This experimental study is a randomized controlled animal design which performed on fifty male Albino mice at weight of approximately 20-25g. Collected from

the Iraqi center for drug control and research. These animals were randomly divided into five groups. Four of these groups were exposed to 1-chloro-2,4-dinitrobenzene (DNCB) to induce atopic dermatitis on the skin located on their dorsal region. A control group consisting of 10 healthy male Albino mice was also included. The animals were housed in a well-ventilated, isolated area within the College of Veterinary Medicine's-University of Baghdad animal shelter. The housing supplied a controlled environment with a room temperature ranging from 20-24°C and a 12-hour light cycle. Before the start of the experiment, the animals were allowed a seven-day period to adapt to the environmental conditions of the room. The treatment course used tacrolimus 0.1% ointment as guideline recommended treatment as well as vinpocetine 5% for comparison, the concentration was prepared similar to other study designs with a treatment period 21 days which it's the optimum for the acute phase.[15] The study was conducted during a period from 1st November 2022 to 1st April 2023. The protocol of the study was approved by Institutional Review Board (IRB) at the College of Medicine-AL Nahrain University (approval no: 202206153). Animals were grouped into the following 5 groups:

Group I: consisted of 10 mice that were apparently healthy.

Group II: included 10 mice that were induced with atopic dermatitis but received no treatment. Group III: The group comprised ten mice that had developed atopic dermatitis. They received topical application of tacrolimus 0.1% ointment once a day at 9:00 AM for 21 days, starting on the seventh day after the induction of the condition.

Group IV: The group consists of ten mice with induced atopic dermatitis. They were treated with topically applied Vinpocetine 5% ointment once a day at 9:00 AM for 21 days, starting from the seventh day after the induction of the condition. Group V: The group consisted of ten mice with induced atopic dermatitis who were given topical application of a vehicle ointment once a day at 9:00 AM for 21 days, starting from the seventh day after the induction of the condition. Various aspects of atopic dermatitis were examined. Firstly, a complete blood count was conducted, which involved assessing different types of white blood cells, including neutrophils, lymphocytes, monocytes, and eosinophils. Secondly, the levels of IL-4 and IL-13 were measured through Immunohistochemistry in mice exhibiting skin lesions caused by atopic dermatitis, and these results were compared to a control group for a thorough analysis. Thirdly, skin lesions originating from atopic dermatitis underwent histological examination, allowing for a comparison with skin lesions from healthy individuals. Finally, to gauge the severity of the condition, an observational scoring system was employed, and this assessment was carried out using a comprehensive blind method by a skilled pathologist. These combined approaches provided a multifaceted evaluation of atopic dermatitis, shedding light on various aspects of the condition's manifestations and severity.[16] Atopic dermatitis was induced by DNCB application to healthy skin of Mouse models. To create a DNCB solution with a concentration of 2%, 100mg of DNCB powder was dissolved in 20ml of a mixture containing acetone and olive oil in a ratio of 3:1 (v/v). Additionally, a DNCB solution with a concentration of 1% was prepared by dissolving 50mg of DNCB powder in 20ml of the same acetone/olive oil mixture. [17] Preparation of Vinpocetine 5% was done by preparing 9.5g of yellow ointment which prepared by melting 0.475g the yellow wax on water bath then 9.025g of petrolatum added and conserved in water bath until uniform thereafter cooling with stirring until congealed afterwards, 0.5g of finely ground Vinpocetine added for each 10g of Vinpocetine 5% ointment.[18]

Vehicle ointment preparation done by preparing 10g of yellow ointment by melting 0.5g the yellow wax on water bath then 9.5g of petrolatum added and conserved in water bath until uniform thereafter cooling with stirring until congealed. [19]. Every blood sample collected in an EDTA tube was analyzed using a compact 5-section hematology analyzer, specifically the BC-5000 model from Mindray. The principle depends on triangle laser scatter, flow cytometry and chemical dye technology. [20]. After 21 days of treatment, skin samples were collected from both groups and examined histopathologically. Microscopic analysis was performed on skin samples from mouse models, and a semi-quantitative scoring system was used with a comprehensive method to grade various the observed conditions encompassing epidermal hypertrophy, hyperkeratosis, parakeratosis, erosion, inflammatory cell infiltration, extracellular edema, and ulceration. The scoring scale ranged from 0 (indicating normal) to 3 (indicating moderate abnormality), with 1+ indicating slight abnormality, 2+ indicating mild abnormality, and 3+ indicating moderate abnormality. [21]. The effect of observations was evaluated by assigning a severity rating. On the 21st day of treatment, the severity of atopic dermatitis (AD) on the dorsal region was assessed and compared between the two groups. The intensity of symptoms, such as redness, dryness, erosion, and swelling, was ranked on a scale of 0 (indicating the absence of symptoms) to 3 (representing severe symptoms). Additionally, the severity was further categorized into four levels: 0 (none), 1 (mild), 2 (moderate), and 3 (severe). To calculate the clinical skin score, the points assigned based on the evaluation of each individual's symptoms were summed. The data analysis was carried out utilizing Microsoft Excel 2013 and SPSS software version 24. Statistical significance was considered significant when the P value was ≤ 0.05. Comparisons between means were made using ANOVA test.

Result

The given information in table (1) presents the results of an ANOVA (Analysis of Variance) test conducted on different treatments (Normal, Induced, Vehicle, Vinpocetine, and Tacrolimus) with respect to WBC. The levels of WBC, neutrophils, lymphocytes, monocytes, and eosinophils were significantly higher in the non-treated induced atopic dermatitis group of mice when compared to the control group. The results of Vehicle treated group showed that these levels were not statistically significantly reduced and did not differ from those of the corresponding non-treated AD-induced group with a p-value greater than 0.05. The results indicated that mice who received Vinpocetine 5% ointment topically showed a statistically significant decrease in these levels after 21 days, with a p-value of less than 0.001, compared to the corresponding levels in the nontreated AD-induced group, there was a statistically significant reduction in the levels of WBCs count in mice that received topically applied Tacrolimus 0.1% ointment compared to the corresponding levels in the non-treated AD induced group.

Table (1): Comparison between the effect of vehicle, vinpocetine and tacrolimus treated group regarding WBC by ANOVA test.

Parameters		Normal (a)	Induced (b)	Vehicle (c)	Vinpocetine (d)	tacrolimus
WBC count ($x10^3$ /*m*l)	Mean	4047.80	14433.00	12271.00	4326.00	4287.00
	P-Value a		***0.000*****	***0.000*****	0.999	0.999
	P-Value b			**0.038**	***0.000*****	***0.000*****
	P-Value c				***0.000*****	***0.000*****
	P-Value d					1.000
Neutrophils count ($x10^3$ /*m*l)	Mean	1544.60	5155.30	4046.80	1179.50	1753.50
	P-Value a		***0.000*****	***0.000*****	0.937	0.995
	P-Value b			0.069	***0.000*****	***0.000*****
	P-Value c				***0.000*****	***0.000*****
	P-Value d					0.689
Lymphocytes count ($x10^3$ /*m*l)	Mean	2311.50	5962.70	6016.90	2932.50	2339.50
	P-Value a		***0.000*****	***0.000*****	0.571	1.000
	P-Value b			1.000	***0.000*****	***0.000*****
	P-Value c				***0.000*****	***0.000*****
	P-Value d					0.619
Monocytes count ($x10^3$ /*m*l)	Mean	80.90	1346.50	989.50	118.20	96.00
	P-Value a		***0.000*****	***0.000*****	1.000	1.000
	P-Value b			0.187	***0.000*****	***0.000*****
	P-Value c				***0.000*****	***0.000*****
	P-Value d					1.000
Eosinophils count ($x10^3$ /*m*l)	Mean	111.70	1958.50	1217.80	95.80	98.00
	P-Value a		***0.000*****	***0.000*****	1.000	1.000
	P-Value b			***0.000*****	***0.000*****	***0.000*****
	P-Value c				***0.000*****	***0.000*****
	P-Value d					1.000

****** Denote highly significant difference at p value ≤0.001

Table (2) shows the respect to various histopathological features (Epidermal thickness, Hyperkeratosis, Parakeratosis, Erosion, Inflammatory cell infiltrate, and Extracellular edema) For each histopathological feature, the table displays the scores or ratings for each treatment based on comprehensive method. (21). The results showed that Vinpocetine treatments led to a statistically significant reduction in hyperkeratosis and inflammation across all studied groups (P<0.001). There were no abnormalities observed in parakeratosis and edema in any of the studied groups, including the Tacrolimus treated group, Vinpocetine treated group and the P values remained constant.

Table (2): Comparison between the effect of Vinpocetine, tacrolimus and vehicle treated group with regard to histopathological changes by (ANOVA test):

Parameters	Normal	Induced	Vehicle	Vinpocetine	Tacrolimus	p-value
Epidermal thickness	0	3	3	1	1	<0.001**
Hyperkeratosis	0	3	3	1	1	<0.001**
Parakeratosis	0	3	2	0	0	<0.001**
Erosion	0	3	1	1	0	<0.001**
Inflammatory cell infiltrate	0	3	3	1	1	<0.001**
Extracellular edema	0	3	2	0	0	<0.001**

****** Denote highly significant difference at p value ≤0.001

Table (3) represents the negative and positive expression of Il-4 and Il-13 which showed that Vinpocetine and Tacrolimus have a suppressing effect on IL-4 and IL-13. This indicates that they can decrease the levels of these cytokines in the context of the study.

Table (3): shows the expression of positive and negative of the immunohistochemistry of IL4 and IL13 inflammatory cells of skin mice.

Parameters	IL-4	IL-13
Normal	Negative	Negative
Induced	Positive	Positive
Vehicle	Positive	Positive
Vinpocetine	Negative	Negative
Tacrolimus	Negative	Negative

The histopathological futures between the study groups are displayed in Figure 1,2,3 and4. The histopathological examination of skin samples taken from the control group of mice revealed normal appearance, as shown in figures (1). However, the skin samples collected from the non-treated induced atopic dermatitis group of mice showed hyperkeratosis, increased epidermal thickness or acanthosis, along with a severe acute inflammatory response, vascular congestion, and focal epidermal sloughing. In figure (2) histopathological scores did not show a statistically significant reduction in the vehicle-treated group, with a p-value greater than 0.001, except for erosion and extracellular edema, and parakeratosis, which demonstrated a significant reduction in the vehicle group with a p-value less than 0.001 after 21 days of topical treatment. The non-treated AD-induced group demonstrated hyperkeratosis, increased epidermal thickness (acanthosis), severe acute inflammatory reaction, vascular congestion, and focal epidermal sloughing. In figure (3) The treated group with tacrolimus 0.1% showed only mild keratosis and mild epidermal thickness, with no inflammatory cells, as compared to the non-treated AD induced group while in figure (4) the group of mice that received topically applied Vinpocetine 5% the scores showed slight keratosis, slight epidermal thickness, slight acanthosis, slight erosion, and edema without any inflammatory cells, with a p value of less than 0.001, compared to the non-treated AD-induced group.

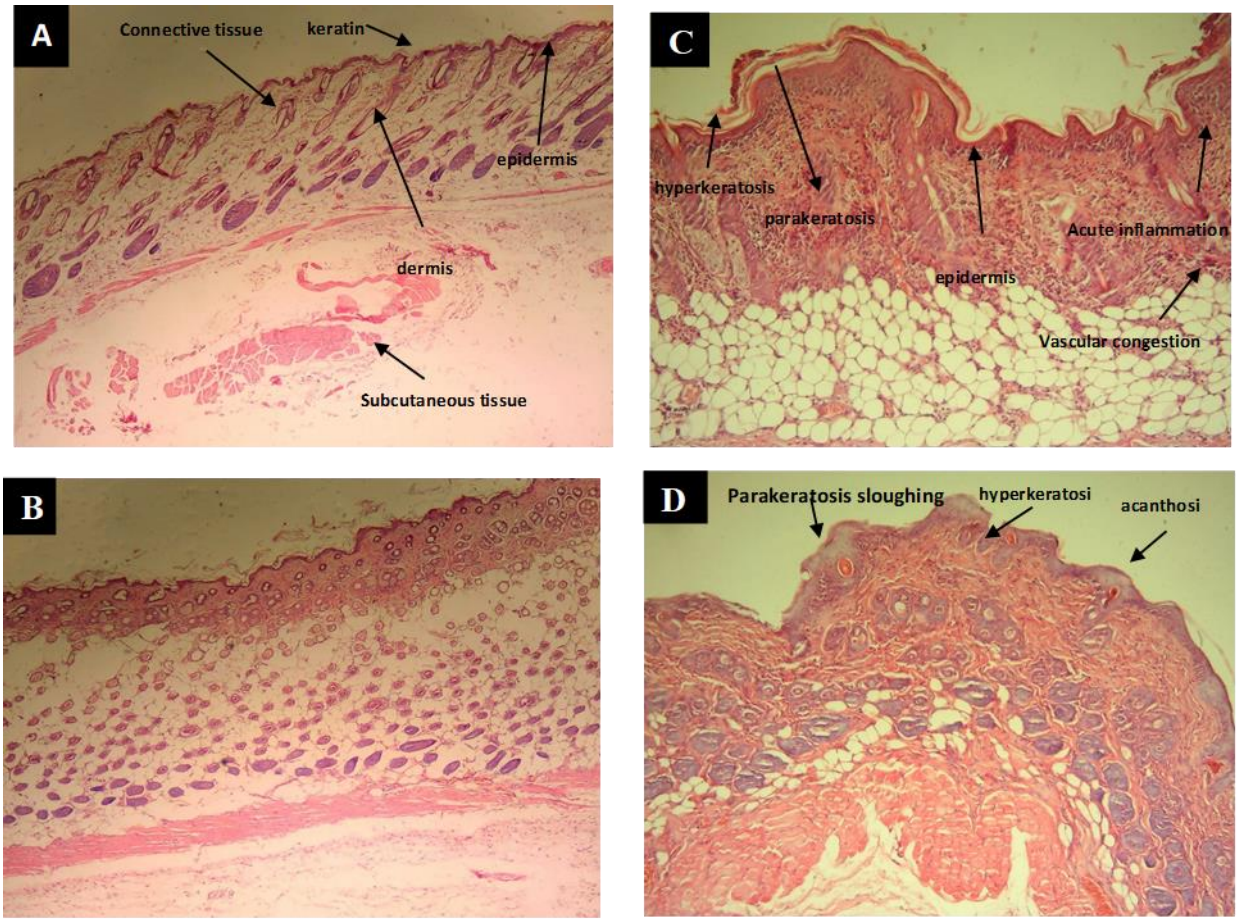
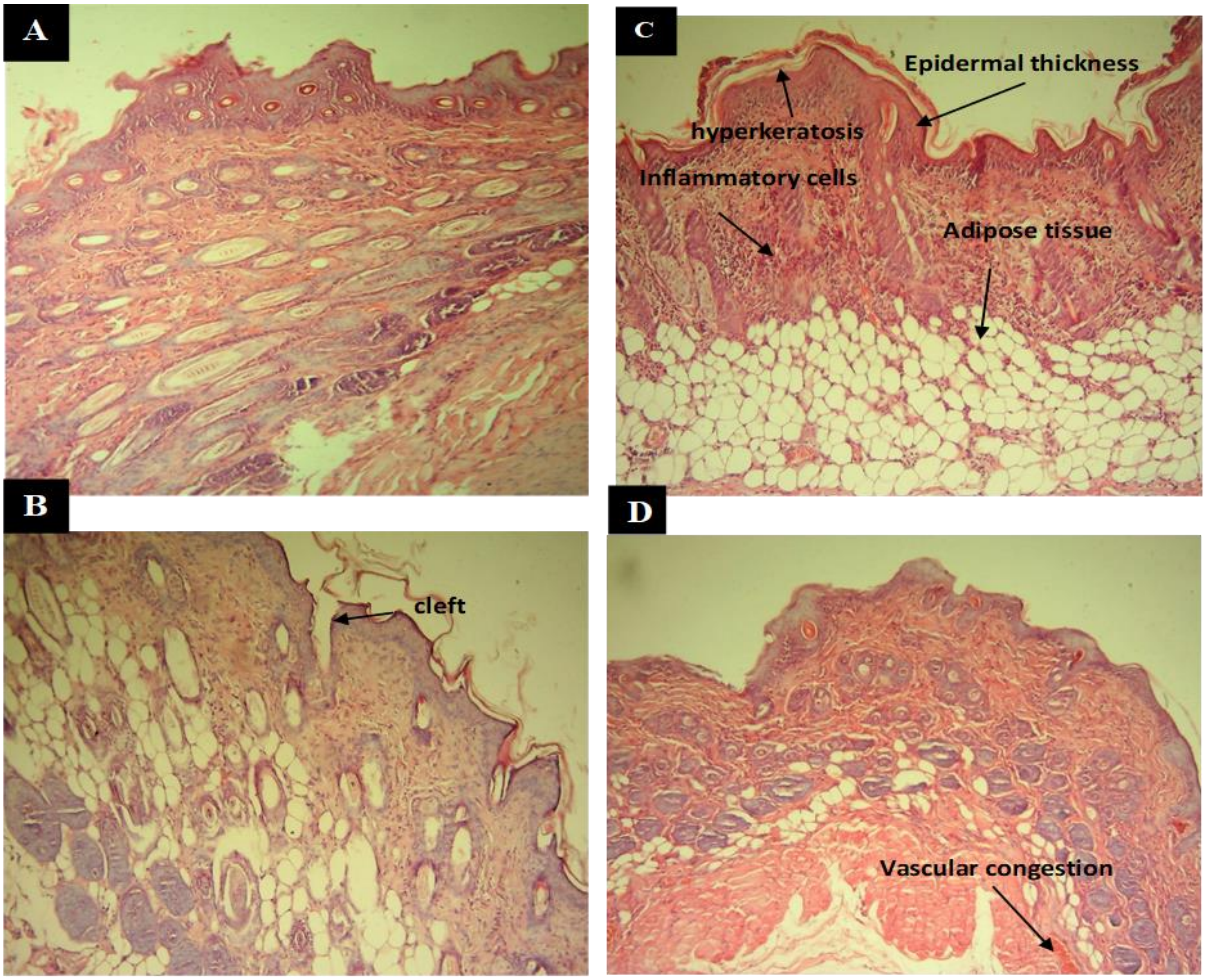
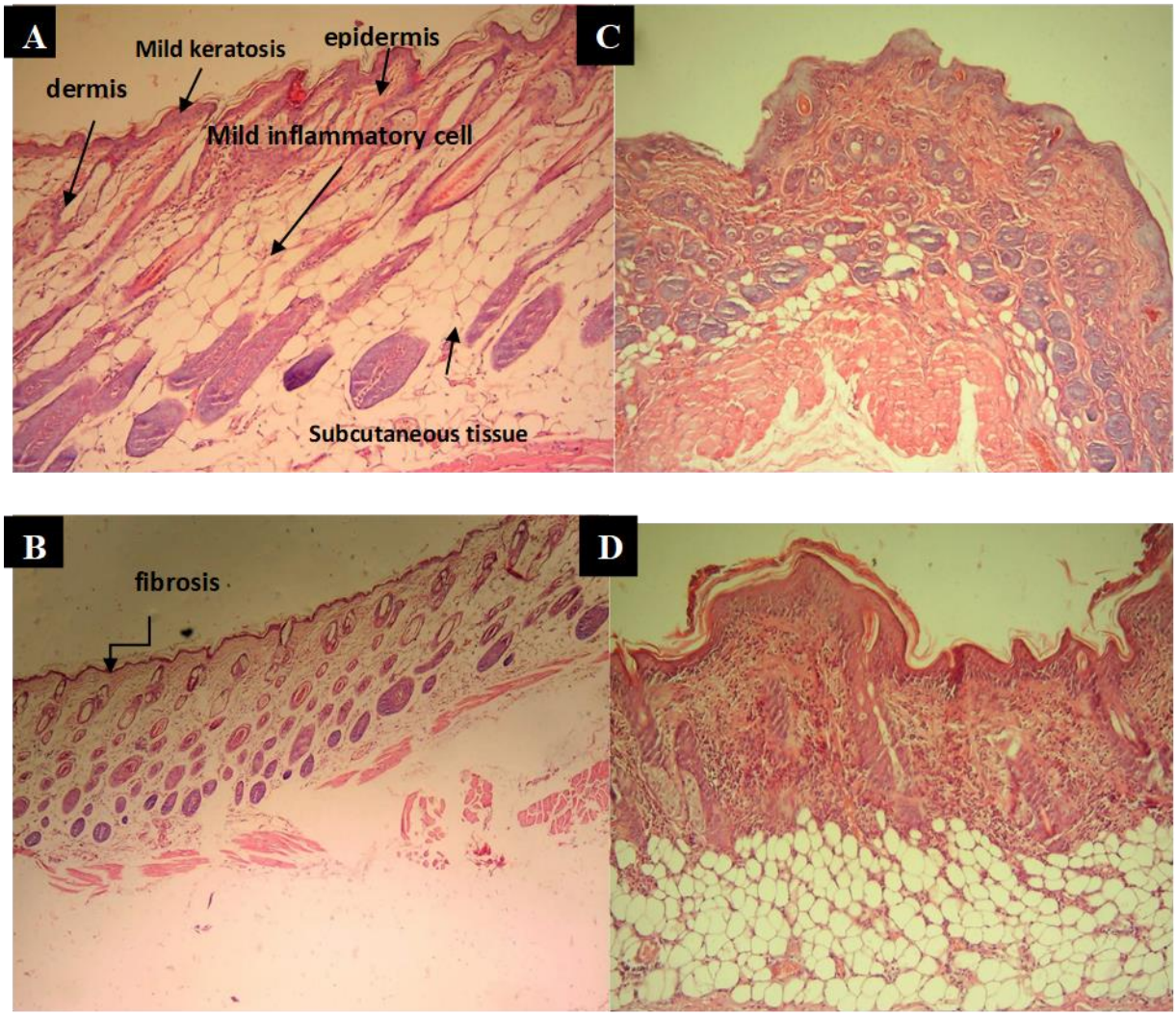


Figure (1): Histopathological section of skin mice in control group (A &B) (10X); compared with histopathological section of induced AD non- treated group (C&D) (10X): ordinary hematoxylin and eosin stain.



Figure(2): Histopathological section of skin mice in atopic dermatitis in vehicle treated group(A&B) (10X) compared with histopathological section of skin mice induced non-treated group (C&D) (10X): ordinary hematoxylin and eosin stain (H&E stain)



Figure(3): Histopathological section of skin mice in atopic dermatitis induced non-treated group (C&D) (10X) compared with histopathological section of skin mice in Tacrolimus 0.1% treated group (A&B) (10X ,4X): ordinary hematoxylin and eosin stain (H&E stain)

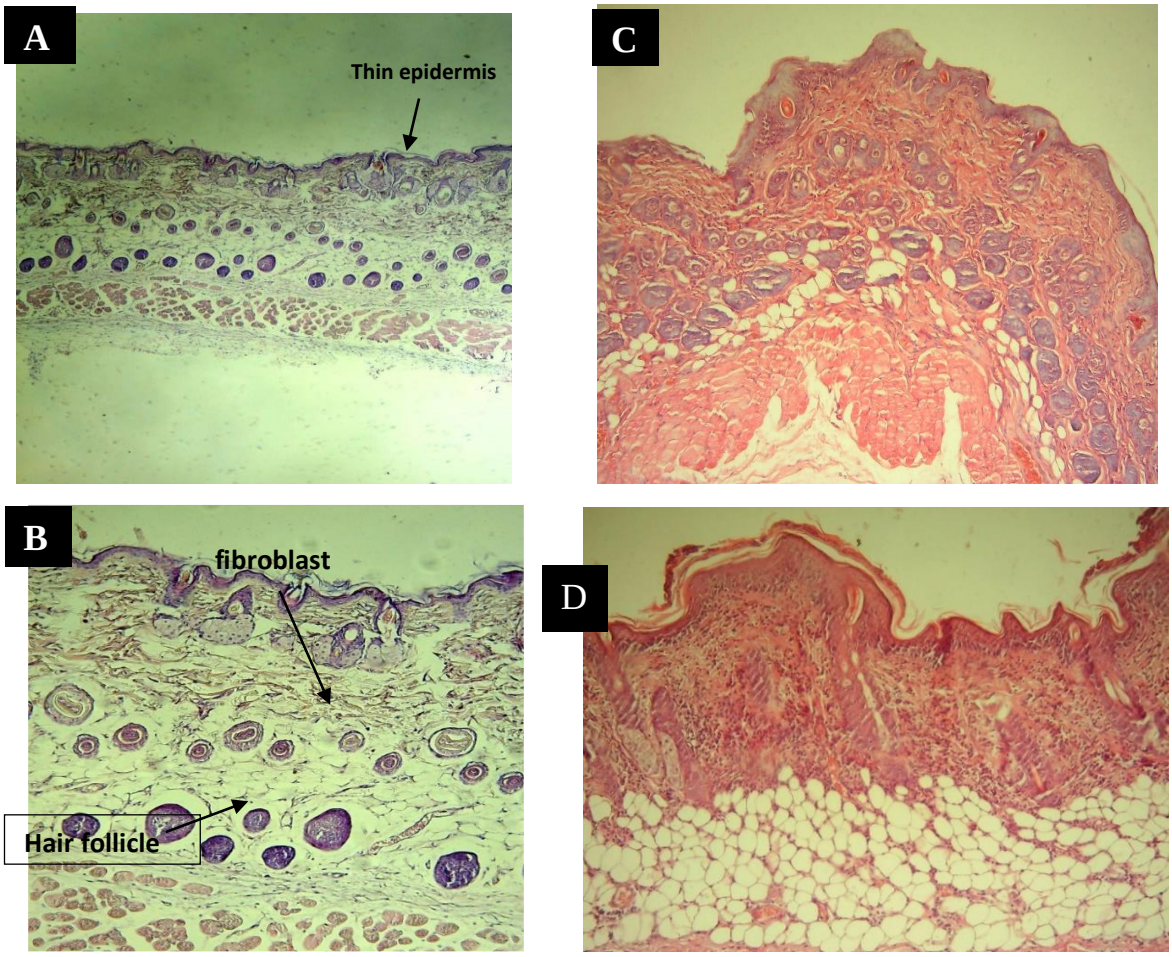


Figure (4): Histopathological section of skin mice in atopic dermatitis induced non-treated group (C&D) (10X) compared with histopathological section of skin mice in Vinpocetine 5% treated group (A&B) (10X,4x) : ordinary hematoxylin and eosin stain (H&E stain)

Discussion

Vinpocetine is a natural compound derived from the periwinkle plant, which has demonstrated anti-inflammatory properties throw an inhibition of phosphodiesterase type-1 (PDE1) analgesics and may affect the inflammation process in a topic dermatitis. [22] in the current study it is found after 21 days of topical vinpocetine 5% treated AD skin lesion induced in mice model highly significant reduction in WBC count and neutrophil count compared with AD induced non-treated group and highly significant decrease monocytes, lymphocytes and eosinophils count as compared to the AD-induced group that wasn't treated, limited studies investigated the effects of vinpocetine ointment on a mouse model of atopic dermatitis.[23] This study found that treatment with vinpocetine resulted in a significant decrease in the levels of eosinophils and lymphocytes, but no significant changes were observed in the levels of WBC, neutrophils, and monocytes. These findings suggest that vinpocetine may have immunomodulatory effects in atopic dermatitis, particularly in reducing eosinophilic inflammation. [15]. The current study showed a highly significant reduction in WBC, neutrophils, lymphocytes, monocytes and eosinophils in comparison between tacrolimus treated group and atopic dermatitis induced non-treated group after 21 days of starting study. In a mouse model of atopic dermatitis induced by 2,4-dinitrochlorobenzene (DNCB), the effectiveness of tacrolimus was investigated. The results showed that the

topical application of tacrolimus 0.1% ointment significantly reduced clinical severity scores and decreased skin thickness in the DNCB-induced atopic dermatitis group compared to the control group. Additionally, the study observed a notable decrease in the number of inflammatory cells, including eosinophils, neutrophils, and mast cells, in the skin of the tacrolimus-treated group when compared to the control group. Recent work obviates the effect of tacrolimus on atopic dermatitis tissue markers, histological and observational severity scores and on the WBC counts of the affected mice. Levels of IL-4 and IL-13 were decreased significantly in topically tacrolimus treated group in comparison with those suffered from atopic dermatitis induced by DNCB which occur after one week of treatment. The assumed mechanism of action of tacrolimus was discussed in several types of research, and they postulated that the primary mechanism of action of tacrolimus is its immunosuppression activity which then subjected to more pro- found studies to elucidate this mechanism precisely.[24] The proposed mechanisms for tacrolimus activity explain its anti-inflammatory and anti-chemotactic role that may provide an acceptable explanation about the other variables and histological scores that reduced after treatment with it for one and two weeks such as the reduction in the counts of total WBCs, neutrophils, eosinophils, and basophils.[25] The present study demonstrated a notable reduction in the immunohistochemistry (IHC) levels of IL-4 and IL-13 in the dorsal region of the skin tissue of mice after 21 days of topical treatment, as compared to the non-treated group with induced atopic dermatitis (AD). Vinpocetine administration inhibited the rise in serum immunoglobulin (Ig) E and IgG1 levels, along with the production of cytokines such as IL-4, IL-5, and IL-13 in mice with AD. This reduction in CBC blood count is attributed to its anti-inflammatory and immunomodulatory effects. Research demonstrated a noteworthy decrease in the expression of both IL-4 and IL-13 in the treatment group compared to the non-treated group. [26] This finding suggests that vinpocetine may exert its beneficial effects on atopic dermatitis by suppressing the expression of these inflammatory cytokines.[11] Regarding Tacrolimus, it works by inhibiting the activity of calcineurin, which is an enzyme that plays a key role in the activation of T-cells and the production of inflammatory cytokines, including interleukin-4 and interleukin-13. By blocking this pathway, tacrolimus reduces the inflammation and immune response that occurs in atopic dermatitis. It also helps to improve the skin barrier function by promoting the differentiation and maturation of skin cells. [27] Observational severity score and histopathological scores in this study were found to be statistically significantly reduced in the group of mice that received topically applied vinpocetine 5% after 21 days of treatment, the effect of vinpocetine 5% ointment resulted in a significant reduction in histopathology score, observational severity score, and parakeratosis, indicating a reduction in skin inflammation and hyperkeratosis. [28] a study was found that vinpocetine reduced renal injury and inflammation in a rat model of sepsis-induced acute kidney injury [23] , Another study also showed that vinpocetine treatment can improve atopic dermatitis-like skin lesions in mice by reducing inflammation and increasing skin barrier function. These findings suggest that vinpocetine may have potential as a therapeutic agent for atopic dermatitis. [15] Regarding the histopathological score, tacrolimus showed a highly significant decrease in the thickness of the epidermis, as well as reductions in hyperkeratosis, parakeratosis, erosion, inflammation, and edema found in tacrolimus treated group with comparison with AD induced non-treated group. Topical application of tacrolimus 0.1% led to a rapid decrease in dermatitis score, inflammation, and pruritus relief. [13] The degree of atopic dermatitis severity was evaluated by

utilizing the observational severity score index, which significantly decreased after treatment with tacrolimus. [29] To ensure that the components of the vehicle did not affect the treatment. This study compared the vehicle treatment group with the induced non-treated group in mice. Histopathology, observational severity index, and the total number of WBC counts did not show any statistically significant differences. Similar results from similar structured studies were reported in who found the histopathological scores between the vehicle-treated group and the untreated group did not differ significantly.[30] These findings suggest that vehicle treatment does not significantly affect the pathological and immunological changes in atopic dermatitis, and highlight the importance of using appropriate control groups in preclinical studies. [31] There were however significant differences in parakeratosis and inflammation, The vehicle-treated group had a lower degree of parakeratosis and inflammation as compared to the untreated group caused by atopic dermatitis. However, it should be noted that the vehicle-treated group still had higher levels of parakeratosis and inflammation compared to the apparently healthy group. This suggests that the vehicle treatment may not completely prevent the development of atopic dermatitis-like skin lesions. [32] A study stated that vehicle treatment in AD shows there was no effectiveness by using *Rumex Japonicus hout* in AD treatment, [33] and slight or no statistically significant changes in the histopathology score and observational severity score there is statistically significant reduction in parakeratosis and inflammation because of it is emollient effect on skin lesion confirm about vehicle role.[34] And positive expression in the IHC of IL-4 and positive expression in IHC of IL-13, this mean there is no significant different with comparison vs. AD induced non treated group; this is confirmed by previous study state vehicle group has no significant difference results when compared with AD induced none treated group. [35] This study had some limitations. The animal tests period was limited due to the lack of proper enviromentation in the animal house which didn't permit long term follow up, also, the study scale was modest, enough to provide an idea about the activity of the used treatment, larger scale study will provide more concrete evidence regrading the benefits of the used treatment.

Conclusion

Topical Vinpocetine 5% ointment, and tacrolimus 0.1% were effective in the treatment of induced AD mouse model through the improvement of histopathological changes and their ability to decrease IL-4 and IL-13, Vinpocetine was effective in treatment of induced AD.

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Conflict of Interest

No conflict of interest was present in the study to declare.

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