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Effect Of Vitisvinifera Extract And Polyphenolicfraction In Treatment Of Induced Atopic Dermatitis Inmice

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Abstract

Background

Itchy skin and pruritus are two of the most common symptoms of Atopic Dermatitis (AD), which is characterized by flares and remissions that occur on a regular basis (Spergel, 2010).

Many theories have been advanced in reference to the development of inflammation that results in AD's disease. The first implies that there is a basic immunological malfunction that results in IgE sensitization, allergic inflammation, and other symptoms. Specifically, the primary immune dysfunction hypothesis proposes that there is an imbalance in T cell subsets, with Th2 cells predominating; this results in the production of type 2 cytokines such as interleukin (IL)–4, IL-5, and IL-13, which leads to an increase in IgE from plasma cells and a secondary epithelial barrier disruption. The second hypothesis posits that there is a fundamental abnormality in the epithelial barrier, which results in subsequent immunologic dysregulation and inflammation. The following medications are often used to treat AD 's disease. Hydrocortisone, triamcinolone, or betamethasone are the most often used steroids. Tacrolimus is animmunomodulator with a broad spectrum of activity.

Vitis vinifera, one of the world's most widely eaten fruits, includes a number of

active components, including organic acids, lipids, and polyphenols.

Grape polyphenols inhibit inflammation by regulating inflammatory pathways and reducing reactive oxygen species (ROS). Grape flavonoids and proanthocyanins combat inflammation better than synthetic drugs.

Introduction

Atopic dermatitis (also known as atopic eczema, AD) is a chronic inflammatory

skin disease that typically begins in childhood and has a variable natural course.

Itching is the disease's hallmark symptom, frequently unrelenting in severe cases ,and contributes to sleep disruption and excoriated, infection.

Cutaneous findings A cute eczematous lesions are characterized by erythematous papulovesicular. While patients with AD may present with a single stage of eczematous lesions, they frequently present with a combination of acute and chronic lesions in multiple areas of the body concurrently or even within the same lesion(Silverberg & Simpson, 2013).

Etiology and Pathogenes is Atopic dermatitis is a complicated familial skin disease caused by interactions among genetic, immune, and environmental risk factors.

I. Decreased skin barrier function A topic dermatitis is linked to a reduction in skin barrier function (Leung, 2016).

II. Genetics More than 80 genes have been associated with AD (Leung & Guttman-Yassky, 2014).The filaggrin gene is found on chromosome 1q21, which contains genes (including involucrin, loricrin, calcium binding proteins and epidermal differentiation complex) (Irvine et al., 2011).

III. Cytokines in atopic dermatitis skin is the presence of thymic stromal lymphopoietin (TSLP) and IL-33 in AD epidermis. TSLP, along withIL-33, are key cytokines secreted byepithelial cells that induce dendritic cells to drive Th0 cells into the Th2 cell differentiation pathway. The expression of IL-4, IL-5, IL-13, IL-25, IL-31, andIL-33 is primarily related with non lesional and acute AD skin lesions(Rich et al., 2018).

Tacrolimus ointmen t 0.03% has been approved for intermittent treatment ofmoderate to severe AD in children aged 2 years and older, with tacrolimus ointment 0.1% approved for use in adults and children 16 years and older(Gada & Laubach, 2009).

Vitis vinifera Grape seed extract is primarily composed of fibers (40%) and complex carbohydrates (29%) as well as oil (16%), proteins (11%) and variable phenols (7 %) (Felhi et al., 2016).

Polyphenols' antioxidant activity includes suppression of ROS generation, inactivating NF-κB, and decreased proinflammatory cytokines and chemokines production, as well as a decrease in Major Histocompatibility (Sun et al., 2015).

MATERIALS AND METHODS

Drugs and chemicals

Tacrolimus ointment 0.1% (Pyxus – India), Vitis vinifera Grape seed (Fractional and Extracted), Distilled water (Iraq) Ethanol 90%(Haymankimia), Chloroform (Sigma-Aldrich Germany), n-Butanol (Haymankimia) Dmso (Chemlab-Belgium), Phthalic anhydride (Germany), Olive oil (Splasz-spain)Vaseline (Alhayat-Syria), Hexane (BDH limited/England), IL-1β IHC(PA5-88078-Invitrogen-China) and IgE IHC(MBS2001397-MyBiosource-USA).

Experimental design: This study included sixty male albino mice of comparable age and weight. The protocols for the animal experiment were carefully reviewed and approved by Al-Nahrain University – Council for the Review of the College of Medicine (approval number 1071 on 1/11/2020). sixty Albino mice at six weeks of age. Mice that had been acclimated to their surroundings for 1 week were randomly divided into six groups.

Group I (10 apparently healthy controls). Five groups (n=50) were given 100 microliters of 5% phthalic anhydride solution (prepared by dissolving phthalic anhydride in 4:1 of freshly mixed acetone and olive oil)(Lee et al., 2014).

The study applied it to the dorsum of the back skin once daily at 9 a.m. for three consecutive days a week for four weeks to generate AD-like symptoms.

Group II (n=10), just 5% phthalic anhydride was utilized as an inducer without treatment. Group III (n=10) this AD induced mice with phthalic anhydride at 9 A.M and treated with Vaseline. Group IV (n=10) this AD induced mice with phthalic anhydride at 9 A.M and treated with polyphenolic fraction 2% ointment. Group V (n=10) this AD induced mice with phthalic anhydride at 9 A.M and treated with whole Vitis vinifera seed extract 2% ointment.Group VI (n=10) this AD induced mice with phthalic anhydride at 9 A.M and treated with tacrolimus 0.1%.

Extraction and fractionation of different active constituents 500 grams of shade-dried pulverized plant materials were first defatted with hexane for 24 hours to remove exclude the fatty materials and then dried at room temperature., then dried and packed in the thimble of the Soxhlet apparatus. In a Soxhlet apparatus, ethanol was used to extract the defatted plant materials at 90 %. When the ethanolic extract is evaporated under reduced pressure by using a rotary evaporator to get a dry extract, it must not be heated above 40 °C. In order to separate the residue, it was dissolved in 500ml of water and partitioned into three fractions: hexane, chloroform, and n-butanol(Harborne, 1998).

Laboratory investigation

Total WBC, neutrophils count, Histopathology, Immunohistochemistry (IHC), and Assessment of observational severity score.

Statistical analysis the data analyzed using (SPSS) version 26. The data presented as mean, standard deviation and ranges. Categorical data presented by frequencies and percentages.(ANOVA) (two tailed)wasused to compare the continuous variables accordingly. Post hoc test (LSD) was run to confirm the differences occurred in parameters between study groups. A level of P – value less than 0.05 was considered significant.

Results

High-performance liquid chromatography (HPLC) examination of polyphenols fraction and whole extraction:

Table (1) Comparison of the standard R_t time with fractional and whole extract

Standard	R_t(standard)	R_t Fraction of polyphenols	R_t whole extraction
Rutin	7.494	7.273	7.636
Quercetin	8.318	8.168	8.241
Kaempferol	8.831	8.845	9.809
Isorhamnetin	10.263	10.574	10.220
Catechin	11.581	-	11.827
Coumarin	14.185	≈ 15.227	14.097

Comparison between healthy group and atopic dermatitis induced without and with treated (Petroleum Vaseline, Polyphenolic fraction, tacrolimus 0.1% ointment, whole extract 2%) group in regard to histopathology score, Immunohistochemistry of IL- 1β and IgE, WBC, neutrophils count, observational severityscore.

histopathology score.

Table 2: Comparison between intact untreated control and all other groups in histopathology.

Study group	Epidermal thickness Mean± SD	Hyperkeratosis Mean± SD	Parakeratosis Mean± SD	Erosion Mean ±SD	Inflammation Mean± SD	Edema Mean ± SD		**P-value**
I	0 + 0	0 + 0	0 + 0	0 + 0	0 + 0	0 + 0	Epidermalthickness	0.001
II	4.0 ± 0	4.0 ± 0	3.2 ± 0.78	2.6 ± 0.96	3.8 ± 0.42	3.7 ± 0.48	Hyperkeratosis	0.001
III	4.0 ± 0	2.25 ± 0.5	2.1 ± 0.99	1.4 ± 0.69	1.6 ± 0.51	2.3 ± 0.82	Parakeratosis	0.001
IV	1.6 ± 0.7	0.9 ± 0.3	0.5 ± 0.52	0 + 0	1.3 + 0.48	0.5 + 0.52	Erosion	0.001
V	1.6 ± 0.7	1.7 ± 0.48	1.3 ± 0.82	0 + 0	1.5 + 0.52	1.1 + 0.31	Inflammation	0.001
VI	1.8 ± 0.6	0.9 ± 0.3	0.48 ± 0.15	0 + 0	1.2 + 0.42	0.8 + 0.42	Edema	0.001

Immunohistochemistry

IgE

Mean of IgE was different significantly among study groups to be highest in group II (3.0, P= 0.001) as shown in table 3

Study Group	**IGE Mean±SD**	**F - Value**	**P-Value**
I	0 + 0	**191.647**	**0.001**
II	3.0 ± 0		
III	2.1 ± 0.31		
IV	1.0 + 0		

V	0.5 + 0.52		
VI	1.0 + 0		

IL-1β

Mean of IL-1β was different significantly among study groups to be highest in groups II and III (3.0, P= 0.001) as shown in table 4

Table 4: Comparison in IgE among study groups

Study Group	IgE Mean±SD	F - Value	P-Value
I	0 + 0	**360.429**	**0.001**
II	3.0 ± 0		
III	3.0 ± 0		
IV	1.3 + 0.48		
V	1.0 + 0		
VI	2.0 + 0		

WBC

Mean of WBC was different significantly among study groups to be highest in group II (10.02, P= 0.001) as shown in table 5

Table 5: Comparison in WBC among study groups.

Study Group	WBC Mean±SD	F - Value	P-Value
I	4.05 + 0.2	**882.14**	**0.001**
II	10.02 ± 0.1		
III	6.98 ± 0.15		
IV	4.36 + 0.43		
V	4.21 + 0.3		
VI	4.49 + 0.33		

Neutrophil

Mean of neutrophil was different significantly among study groups to be highest in group II (15.2, P= 0.001) as shown in table 6

Study Group	Neutrophil	F - Value	P-Value

	Mean±SD		
I	0.83 + 0.02	**3501.22**	**0.001**
II	15.2 ± 0.44		
III	9.86 ± 0.4		
IV	1.14 + 0.4		
V	0.94 + 0.2		
VI	1.56 + 0.31		

Observational severity score

Mean of observational severity score was different significantly among study groups to be highest in group II (11.0, P= 0.001) as shown in table 7

Study Group	Observational severity score Mean±SD	F - Value	P-Value
I	0 ± 0	**284.79**	**0.001**
II	11.0 ± 0.7		
III	6.8 ± 1.2		
IV	2.5 ± 0.7		
V	4.4 ± 0.7		
VI	2.4 ± 0.5		

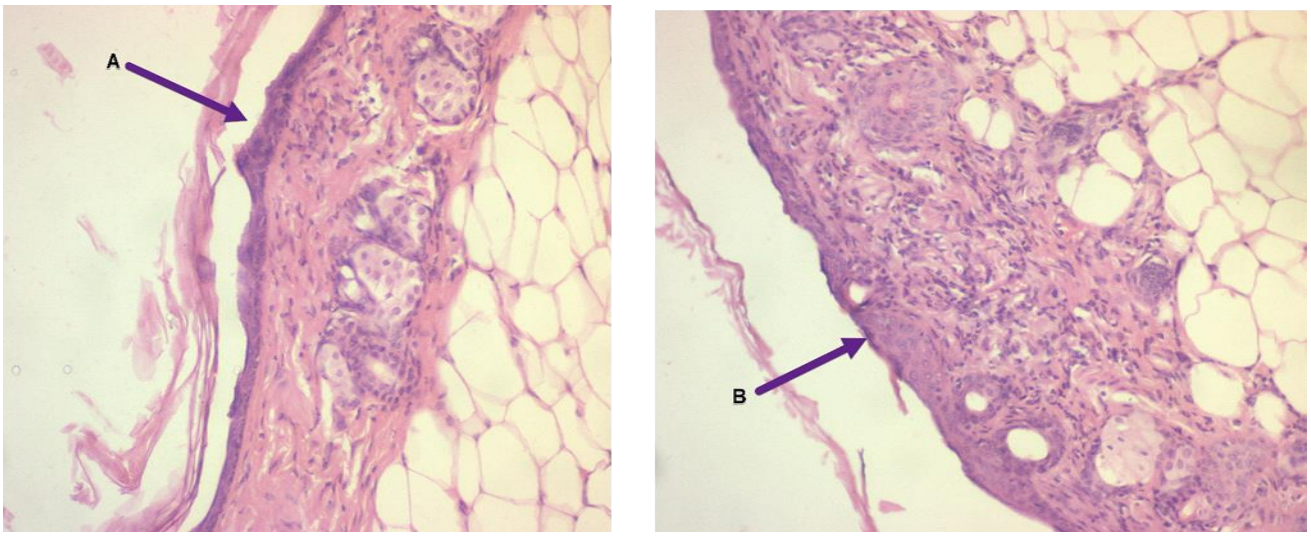
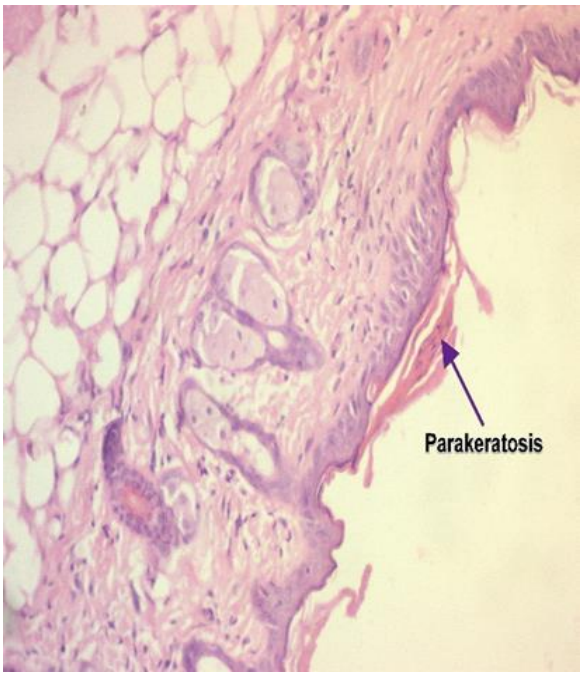
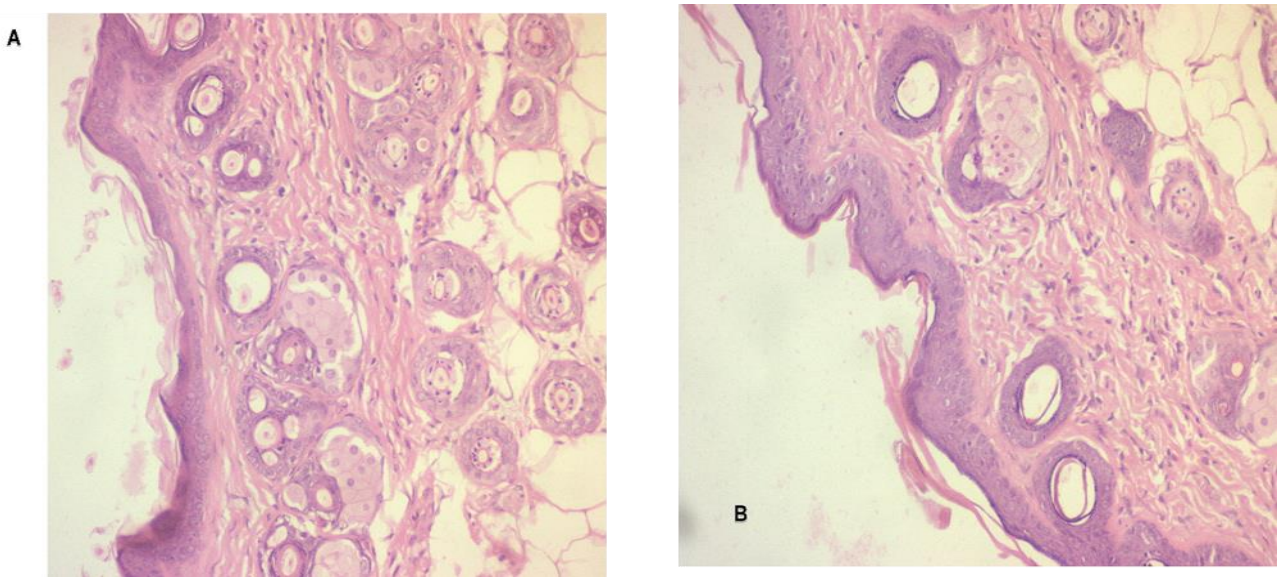


Figure (1): Histopathology section through skin showing healthy skin (A) and atopic dermatitis induced non-treated skin (B) in which there is evidence of sever epidermal thickening(arrow), sever hyperkeratosis and inflammation; 20x; Hematoxylin and eosin stain.



(A) (B)

Figure (2): Tacrolimus has been shown to affect parakeratosis



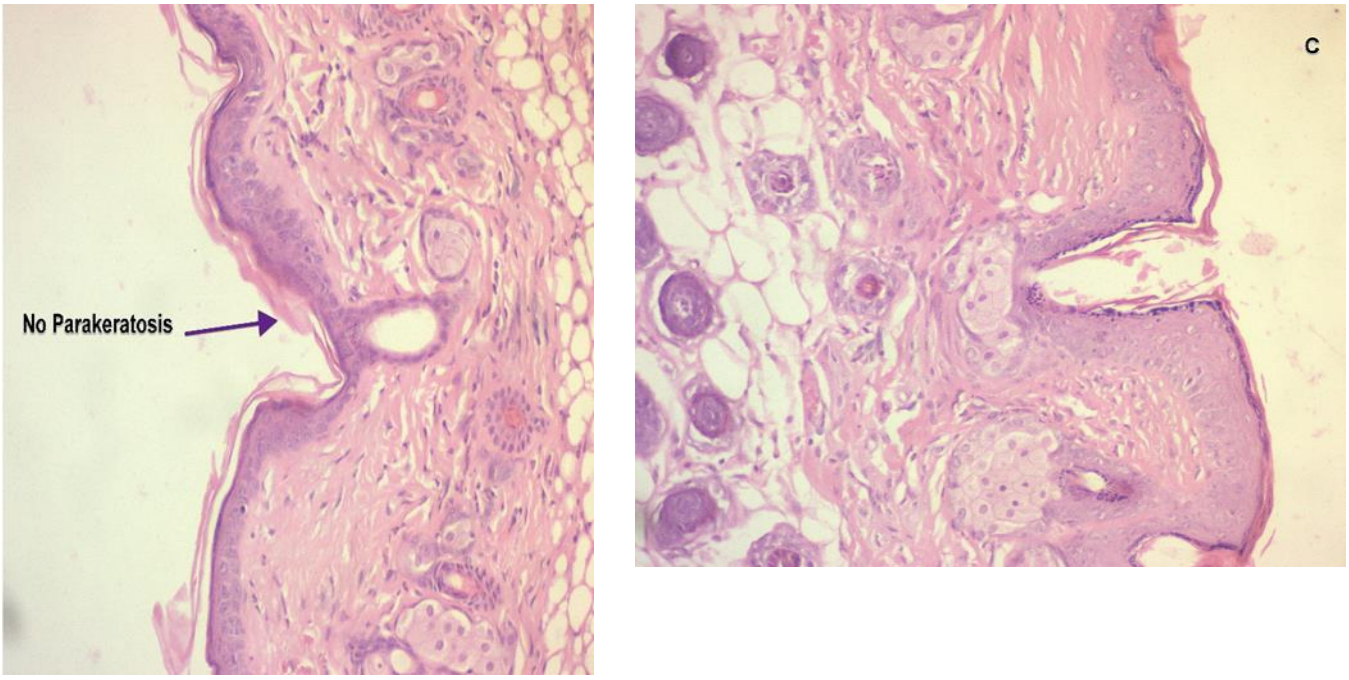


Figure (3): (A) Fractional (B) whole extract (C) Vaseline

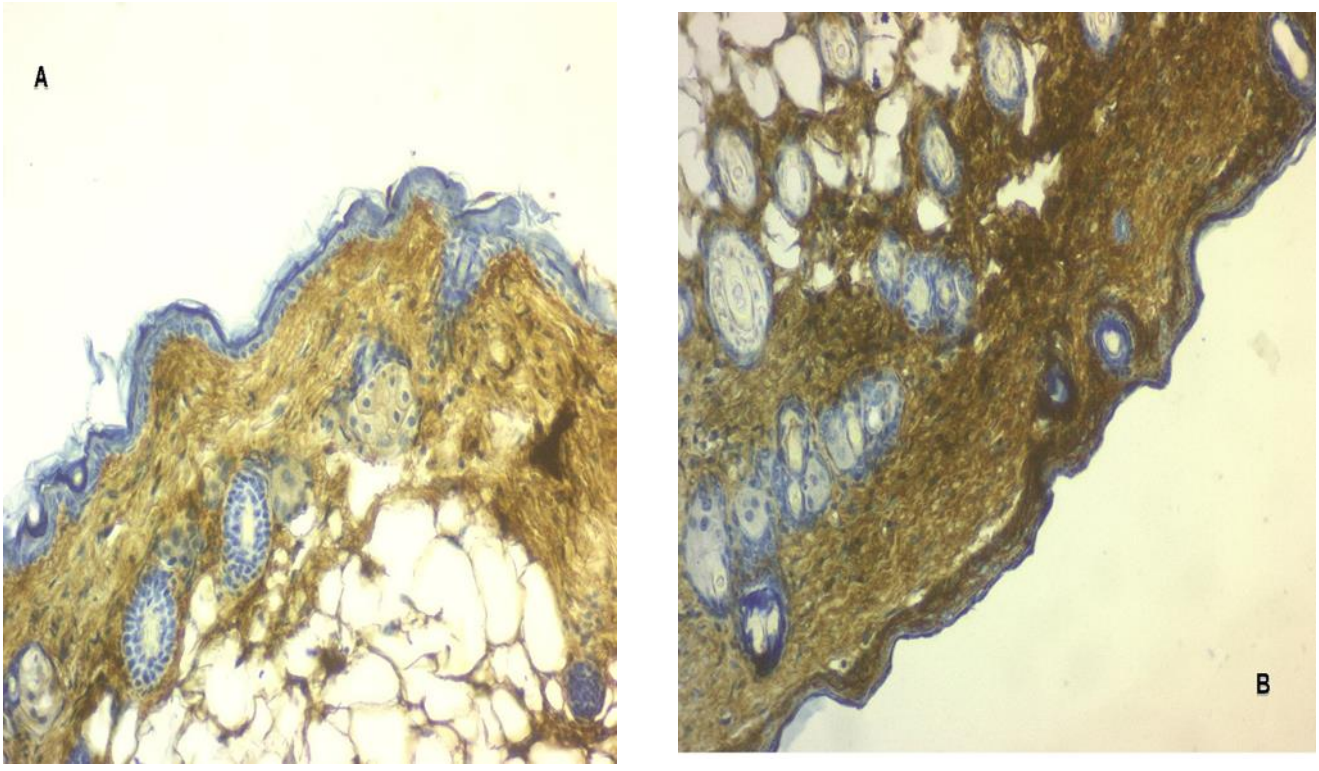


Figure (4): Immunohistochemistry of IgE score of healthy groups (A) (20x) and atopic dermatitis induced non-treated group (B) (20x).

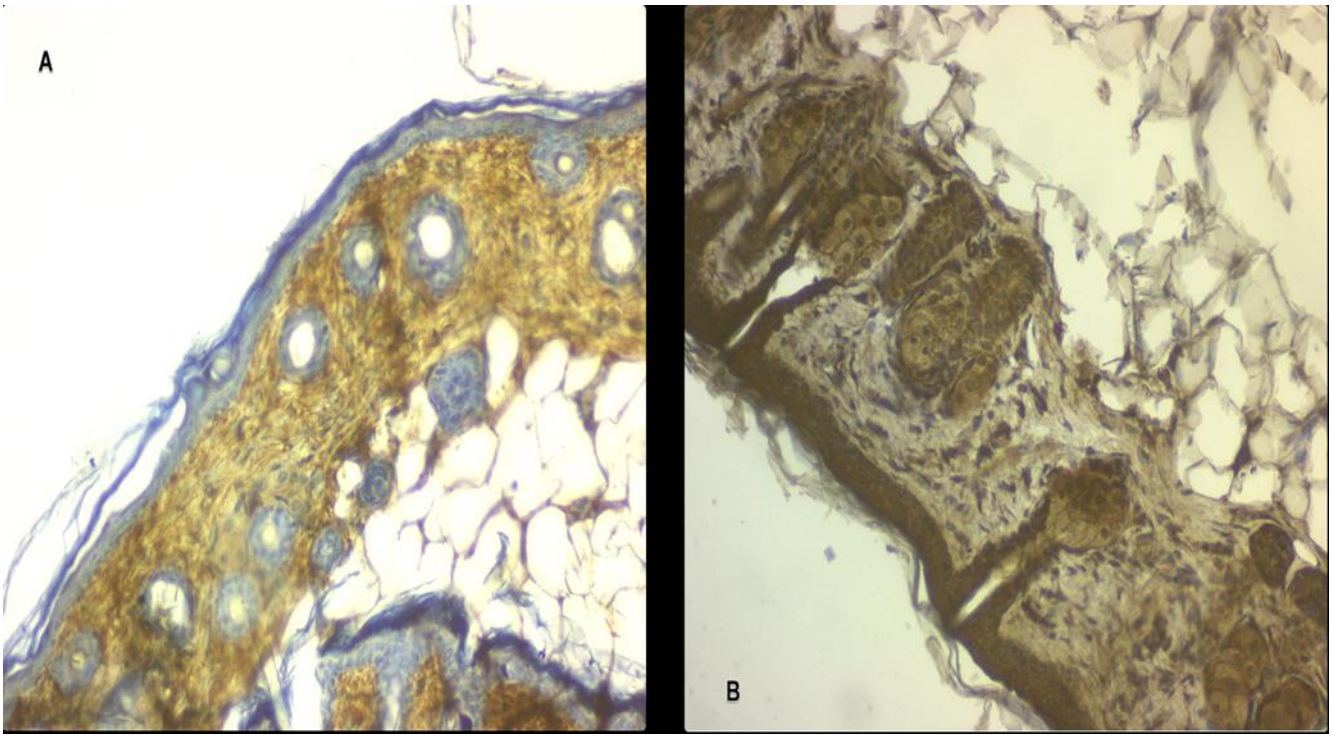


Figure (5): Immunohistochemistry of IL-1β score of healthy groups (A) (20x) and atopic dermatitis induced non-treated group (B) (20x).

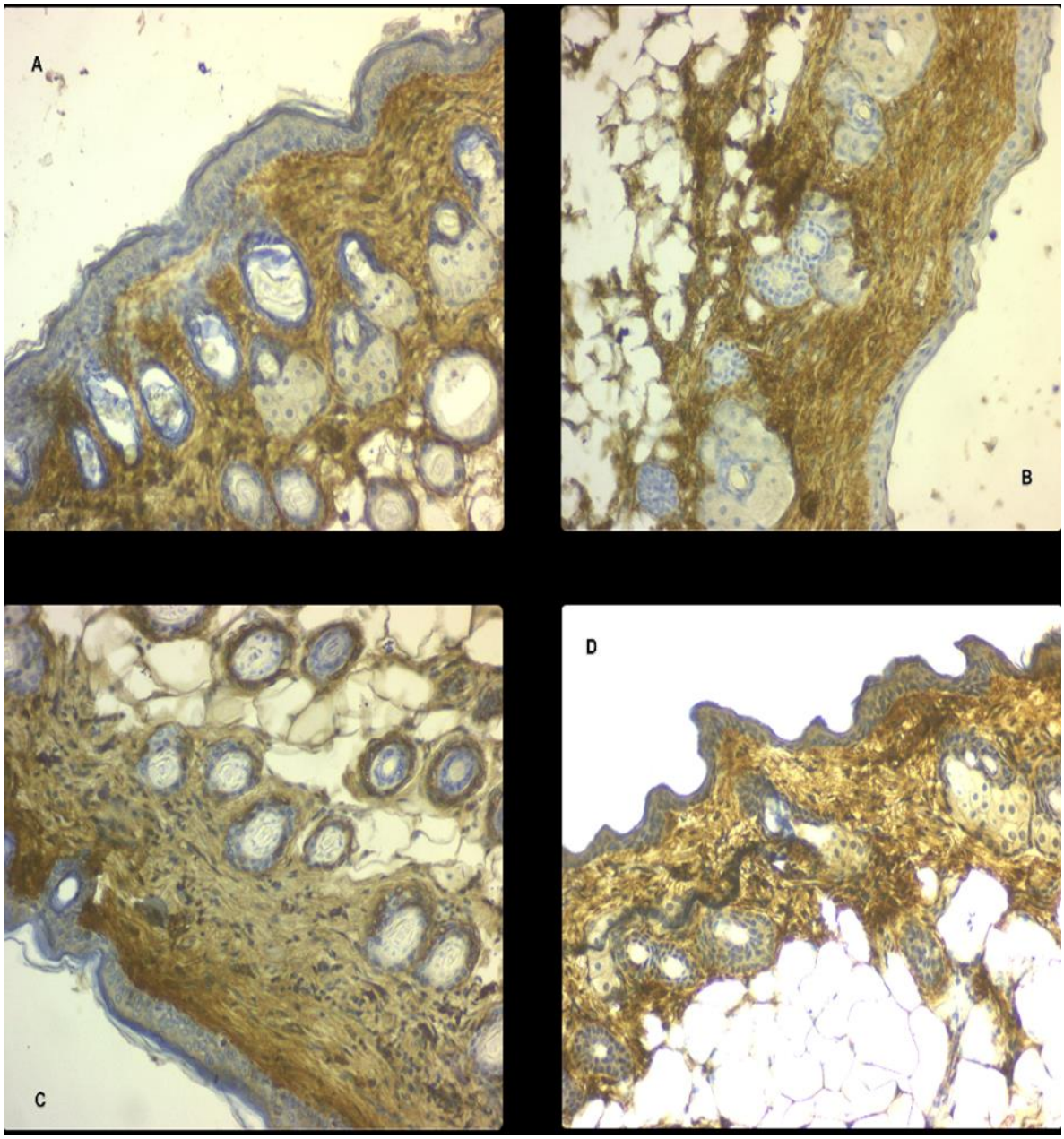


Figure (6): Immunohistochemistry of IgE score of Tacrolimus (A) (20x), Fractional (B) (20x), whole extracted (C) (20x) and Vaseline (D) (20x).

Discussion All included groups evaluated first for its weight to exclude weight related

changes in response to the 4 weeks/3 days a week application of topicaltreatment; no statistically significant differences are found regarding miceweight after a month of treatment. These finding were in concordance to a studyby kwak et al where topical phthalic anhydride used to induce AD in healthymice(Kwak et al., 2013).

Histopathological examination of included cases revealed that: thickness of the epidermal layer was highest in Atopic induced groups that received no treatment and /or had topical petroleum Vaseline only. While lowest in healthy

group. Application of phthalic anhydride assessed before and found to induce AD like reaction when applied on the skin of healthy mice.

Epidermal thickness resulted from hyperkeratosis, parakeratosis and spongiosis; in addition to the heavy inflammatory cells infiltrate confirmed histopathologically with H&E stain performed(Jiang & Ma, 2017).No differences in epidermal thickness in all groups that received: topical polyphenolic fraction 0.25%, topical tacrolimus ointment 0.1%, and topical whole extract of Vitis vinifera 2%. Although the differences were significant between (tacrolimus, pure polyphenol & crude whole extract) treated groups and eczematous group; indicating the inflammatory and proliferative mitigating effects of pure and crude Vitis vinifera seeds is as effective as tacrolimus.

Regarding hyperkeratos is that hyperkeratosis was lower in both pure polyphenols treated group and crude Vitis vinifera extract than tacrolimus treated group.

Parakeratosis It was lowest in both groups treated by whole grape seeds extracts and purified polyphenol fraction groups even lower than tacrolimus treated.

Edema Anti-inflammatory agent like tacrolimus decreases it, interestingly its effects is comparable to purified polyphenol and crude whole grape seed extract with no significant statistical differences. This is comparable to a study by Abdullah et al who found that grape seeds extract is being used for preventing edema because of properties of its ingredients especially proanthocyanidin, which have the ability to inhibit the proinflammatory cytokines, COX-1 and 2, 5-lipoxygenase that increasing the plasma levels of prostacyclin and 6-keto-prostagladin. It is also significantly decreasing leukotriene and prostacyclin concentrations(Abd-Allah et al., 2018).

Erosion No significant differences are found between polyphenol, whole grape seed extract and tacrolimus treated groups.

As a conclusion to histopathology severity scores despite Tacrolimus ointment proved and well-known ability in reducing dermatitis and ameliorating histopathological changes like decrease epidermal thickness and inflammatory infiltration(Yamamoto et al., n.d.).

Assessing IgE score between these groups showed higher result in AD group followed by petroleum only treated group then polyphenol group and whole extract with no differences between them but lower in tacrolimus treated group. Tacrolimus inhibit IgE level through the suppression of T cellsresponse to foreign antigens, this has been proved in a study by(He et al., 2019).

Our study proved that too as the lower concentration was in tacrolimus treated cases IgE levels is lower in grape seeds extract pure and crude than atopic treated group.

Interleukin 1β: is elevated in both AD and petroleum treated groups equally. Ithas also found to be high in whole grape seeds extract traded and polyphenol groups, although higher in former than latter.

Blood assessment of mice showed high leukocytes count in AD followed by petroleum then the whole Vitis vinifera extract groups. No significant differences were found between healthy, tacrolimus, pure polyphenol treated groups. Similar finding was detected when neutrophil count was evaluated. In a study by(Caproni et al., 2007).

this study showed that the AD observed severity of tacrolimus treated group is worse than polyphenol fraction and whole extract of Vitis Vinifera.

Conclusion All the therapeutic agents were effective at reducing epidermal thickness, although fractional was superior to Tacrolimus, both the fractional polyphenol and whole Vitis vinifera extract agents had less hyperkeratosis than the tacrolimus treatment group.

Whole grape seed extracts and purified polyphenol fraction groups have the ability to heal the epidermis back to its normal quality.Tacrolimus's effects in edema are comparable to those of fractionated polyphenols and whole Vitis vinifera extract.Vitis vinifera as (fractional and whole extract) is comparable in its effect to tacrolimus on IgE in the atopic dermatitis mice model.

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