

Effects Of Topical Petroleum Ether and Ethyl Acetate Fractions from Grape Seed Extract on Imiquimod-Induced Psoriasis Like Skin Inflammation in Mice

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Abstract:

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Background: Grape seed is a natural herb with many suggested pharmacological effects related to many biologically active compounds in its extract and fractions. Grape seed pharmacological effect is investigated in mice psoriasis like model.

Methods: A thirty male albino mice, six weeks' age used in this research and were divided into five groups. Group I apparently healthy. Group II induction group, imiquimod 5% cream once daily used at the back skin of the animal for five days. Group III, IV and V treatments groups. Group III clobetasol ointment 0.05%, group IV Grape seed extract and group V Grape seed petroleum ether fraction for five days. **Results:** A high significant increase was found between the apparently healthy and imiquimod induced group for all of the investigated parameters except munro abscess was significant increase. For the grape seed extract group, it was found A significant difference in immunohistochemistry score of IL17, also significant decrease in vascular endothelial growth factor (VEGF) with high significant decrease of other immunohistopathology measurement like parakeratosis. **Conclusion:** Grape seed extract may have a role in imiquimod-induced psoriasis like skin inflammation in mice treatment which make it a future candidate therapy for psoriasis.

Keywords: psoriasis, vitis vinifera, grape, imiquimod.

تأثير أجزاء الأثير البترولي والاثيل اسيتيت المستخلص من بذور العنب على التهاب الجلد المشابه للصدفية في الفئران المحفزة بالايميكيومود.

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الخلاصة:

تعد بذور العنب من الاعشاب الطبيعية المصاحبة للكثير من التأثيرات الدوائية الممكنة والتي تعود الى العديد من المركبات الفعالة حيويًا في مستخلص و اجزاء النبات. تم بحث استعمالها في الالتهاب المشابه للصدفية في الفئران باستعمال مختلف القراءات. حيث تم في هذا البحث استخدام ثلاثون فأر أمهق ذكر بعمر ستة اسابيع وتم تقسيمهم الى خمسة مجاميع في هذا البحث. المجموعة الاولى طبيعية. المجموعة الثانية محفزة باستخدام الايميكيومود 5% مرة يومياً يوضع على جلد ظهر الفار لمدة 5 ايام متتالية. المجموعة الثالثة والرابعة والخامسة يستخدم نفس المحفز وجرعته وبعد 3 ساعات يستخدم العلاج. المجموعة 3 كلوبيتازول مرهم 0.05% والمجموعة 4 مستخلص بذور العنب والمجموعة الخامسة جزء الاثير البترولي من بذور العنب لمدة 5 ايام متتالية. بينت الدراسة زيادة معنوية بين المجموعة الطبيعية والمجموعة الثانية المحفزة لكل القراءات والتحليل. وجدت الدراسة ان مجموعة مستخلص بذور العنب نقصان معنوي في قراءات الكيمياء الهستولوجية المناعية لانترولوكين 17 , اضافة الى نقصان معنوي في عامل النمو البطاني الوعائي مع نقصان كبير معنوي في قراءات

الهستوباثولوجيا الاخرى مثل نظير التقرن. تستنتج الدراسة ان مستخلص بذور العنب له دور علاجي في التهاب الجلد المشابه للصدفية في الفئران المحفزة بالايبيكويمود مما قد يجعله مرشح مستقبلي لعلاج الصدفية.

الكلمات المفتاحية: الصدفية، العنب، الايبيكويمود

Introduction:

Vitis vinifera is the mostly used herbal product¹. It is well known that many topical side effects occurred due to the use of corticosteroids. Those side effects were shown to be common and occur concomitantly with the beneficial effect of corticosteroids. Atrophy of the skin, perturbed cicatrization and stretch marks mostly occur. Hypertrichosis, perioral dermatitis, steroid acne, telangiectasia and erythema may occur². Vitis vinifera compose of different phytochemical constituents in the root, cane, stem, leaf, fruit, seed, skin, and pomace. The important compounds are phenolic, flavonoids, aromatic acids, stilbenoids, and proanthocyanidins^{3,4,5}. Research revealed that grape seed extract had antioxidant, anticancer, anti-inflammatory, anti-apoptotic, anti-necrotic, and anti-carcinogenic activity^{6,7,8}. Grape seed extract also have various skin regenerating properties which helped to reduce skin ageing, improve skin appearance, and facilitate wound healing^{9,10}.

Psoriasis is an inflammatory cutaneous disease developed with a chronic scale, and stiff erythematous skin. Psoriasis histologically characterized by proliferation of cutis blood vessels, epidermal hyperplasia and leukocytes inflammatory infiltration that mainly occur in dermis¹¹.

The onset of the psoriasis starts with unknown gene defect or antigen that causes activation of T lymphocyte which depend on the binding of it with antigen presenting cell. T cells will express the T cell receptor (TCR) that recognize the previously presented antigen. The antigen will stimulate the activation causes the naïve T-cells transforming to antigen specific cell. Antigen specific cell can develop to a memory cell that pass around the body. Increase Th-1 immunity response,

neutrophil migration, adhesion molecule up-regulation, keratinocyte reproduction, epidermal hyperplasia, and angiogenesis occur due to the effect of cytokines that secreted from the activated T cells like TNF- α , IL-1, IL-6, IL-17, IL-23, EGF and others¹².

This research is done to investigate the pharmacological effect of grape seed extract and fractions on imiquimod induced psoriasis like inflammation in mice.

Material and Methods

Study Design:

A Randomized, prospective animal study, placebo-controlled research done in the section of pharmacology and toxicology, college of pharmacy, Al-Nahrain University. Thirty albino male, six weeks' age mice were divided randomly into five groups (each of six animals). Group I apparently healthy. Group II induction group, Imiquimod 5% cream (Glenmark) once daily was applied at the back skin of the animal for five days¹³. Group III, IV and V are treatment groups that used the same inducer and dose then after three hours used treatment application. Group III clobetasol ointment 0.05%¹⁴(SDI), group IV Grape seed extract, dose 50mg/kg once daily¹⁵ and group V Grape seed petroleum ether fraction, dose 30mg/kg once daily¹⁶ were used after three hours of using imiquimod 5% cream for five days. The protocols of the study authorized by AL- Nahrain University- college of pharmacy ethical committee (Number 1210, date of approval 18-November-2021).

Preparation of grape extract and fraction:

Vitis vinifera, grapes were collected from local market, and seeds were detached from the flesh manually. The seeds were air dried for 1 week. Two hundred fifty grams of the

dried seeds were pulverized into course powder using mechanical grinder¹⁷. The course powder was extracted with 85% ethanol using soxhlet apparatus for 8hrs. The alcoholic extract was then filtered, and the solvent was evaporated via rotary evaporator. Part of the crude extract was partitioned with petroleum ether (40-60) and ethyl acetate for solvent extraction. The crude and petroleum ether fraction were selected for this study due to high yield^{17,18,19}. Each of them mixed with vaseline to make the required above dose.

Immunohistochemical Evaluation:

Immunohistochemistry (IHC) is done using IHC kit of interleukin -17 (IL-17), Vascular endothelial growth factor (VEGF), and Transforming growth factor (TGF beta 1) catalog number ab193955 (Abcam, USA), ab1316 (Abcam, USA), and SL0086R (Sunlong Biotech, China) respectively. The procedure of IHC was initially evaluated and established by the department of pathology, consultant center, college of medicine, AL – Nahrain University. The procedure is done according to the catalog’s protocols.

Histopathological Evaluation:

At the end of the research, mice were sacrificed using chloroform as inhalant anesthetic. Tissue of the skin was collected from the back of the animal and buffered formaldehyde used for storage²⁰. Scoring system was used for mouse model evaluation histopathology (Munro abscess (1.5), hyperkeratosis (0.5), parakeratosis (1), lengthening of rete ridges (0.5-1.5), acanthosis (1), papillary papillae congestion (1), and dermis lymphocytic infiltrate (0.5-1.5)) each given number according to scale by Baker²¹.

Statistical Analysis:

Statistics used to analyze data by use of SPSS version 26 to find median and range in addition to Mann Whitney test and Kruskal Wallis and take into consideration it is significant result if probability value (p) was less or equal to 0.05. High significant if p value was less or equal to 0.001²².

Results:

Table (1): Comparison of Immunohistochemistry parameters in mice skin

Parameter		Healthy group N=7	Psoriasis group N=6	Clobetasol group N=6	Grape extract group N=6	Grape petroleum ether group N=6
IL-17	Median (Range)	0 (0-1)	3 (1-3)	2 (1-2)	1 (1-2)	1 (1-2)
	P value _a		0.002	0.008	0.035	0.035
	P value _b			0.041	0.026	0.026
	P value _c				0.394	0.394
	P value _d					1.000
	P value _e			0.427		
VEGF	Median (Range)	1 (1-1)	2.5 (2-3)	2 (2-3)	1.5 (1-2)	1 (1-1)
	P value _a		0.001	0.001	0.138	1.000
	P value _b			0.699	0.026	0.002

	P value _c				0.065	0.002
	P value _d					0.180
	P value _e			0.003		
TGF B	Median (Range)	1 (1-1)	3 (2-3)	2 (1-2)	2.5 (2-3)	2.5 (2-3)
	P value _a		0.001	0.051	0.001	0.001
	P value _b			0.026	0.699	0.699
	P value _c				0.065	0.065
	P value _d					1.000
	P value _e			0.041		

a: p value by comparison healthy group with each other group by Mann Whitney test

b: p value by comparison induced group with each treatment group by Mann Whitney test

c: p value by clobetasol with each grape group by Mann Whitney test

d: p value by comparison between two grape groups by Mann Whitney test

e: p value by comparison among treatment groups by Kruskal Wallis test

Data are presented as median (Range), (N) number of animals.

Significant change when P value ≤ 0.05

Highly significant change when P value ≤ 0.001

Table (2): Comparison of histopathological parameters in mice skin

Parameter		Healthy group N=7	Psoriasis group N=6	Clobetasol group N=6	Grape extract group N=6	Grape petroleum ether group N=6
Munro abscess	Median (Range)	0 (0-0)	2 (0-2)	0 (0-0)	0 (0-0)	0 (0-0)
	P value _a		0.008	1.000	1.000	1.000
	P value _b			0.015	0.015	0.015
	P value _c				1.000	1.000
	P value _d					1.000
	P value _e			1.000		
Hyperkeratosis	Median (Range)	0 (0-0)	0.5 (0.5-0.5)	0 (0-0)	0.5 (0.5-0.5)	0.25 (0-0.5)
	P value _a		0.001	1.000	0.001	0.138
	P value _b			0.002	1.000	0.180
	P value _c				0.002	0.180

	P value _d					0.180
	P value _e			0.003		
Parakeratosis	Median (Range)	0 (0-0)	1 (1-1)	0 (0-1)	0 (0-1)	0 (0-0)
	P value _a		0.001	0.366	1.000	1.000
	P value _b			0.065	0.002	0.002
	P value _c				0.394	0.394
	P value _d					1.000
	P value _e			0.119		
Lengthening of rete ridges	Median (Range)	0 (0-0)	1.5 (1.5-1.5)	1.5 (0-1.5)	1.5 (0-1.5)	0 (0-1.5)
	P value _a		0.001	0.051	0.051	0.628
	P value _b			0.394	0.394	0.015
	P value _c				1.000	0.180
	P value _d					0.180
	P value _e			0.151		
Acanthosis	Median (Range)	0 (0-0)	0.5 (0.5-0.5)	0.5 (0.5-0.5)	0.5 (0.5-0.5)	0.5 (0.5-0.5)
	P value _a		0.001	0.001	0.008	0.001
	P value _b			1.000	0.699	1.000
	P value _c				0.699	1.000
	P value _d					0.699
	P value _e			0.368		
Papillary congestion	Median (Range)	0 (0-0)	0.5 (0.5-0.5)	0.5 (0.5-0.5)	0.5 (0.5-0.5)	0.5 (0.5-0.5)
	P value _a		0.001	0.001	0.001	0.001
	P value _b			1.000	1.000	1.000
	P value _c				1.000	1.000
	P value _d					1.000
	P value _e			1.000		

Dermis lymphocytic infiltrate	Median (Range)	0 (0-0)	2 (2-2)	0.75 (0.5-1)	0.5 (0-0.5)	0.25 (0-0.5)
	P value _a		0.001	0.001	0.008	0.138
	P value _b			0.002	0.002	0.002
	P value _c				0.093	0.026
	P value _d					0.394
	P value _e			0.023		

a: p value by comparison healthy group with each other group by Mann Whitney test
 b: p value by comparison induced group with each treatment group by Mann Whitney test
 c: p value by clobetasol with each grape group by Mann Whitney test
 d: p value by comparison between two grape groups by Mann Whitney test
 e: p value by comparison among treatment groups by Krustal Wallis test
 Data are presented as median (Range), (N) number of animals.
 Significant change when P value ≤ 0.05
 Highly significant change when P value ≤ 0.001

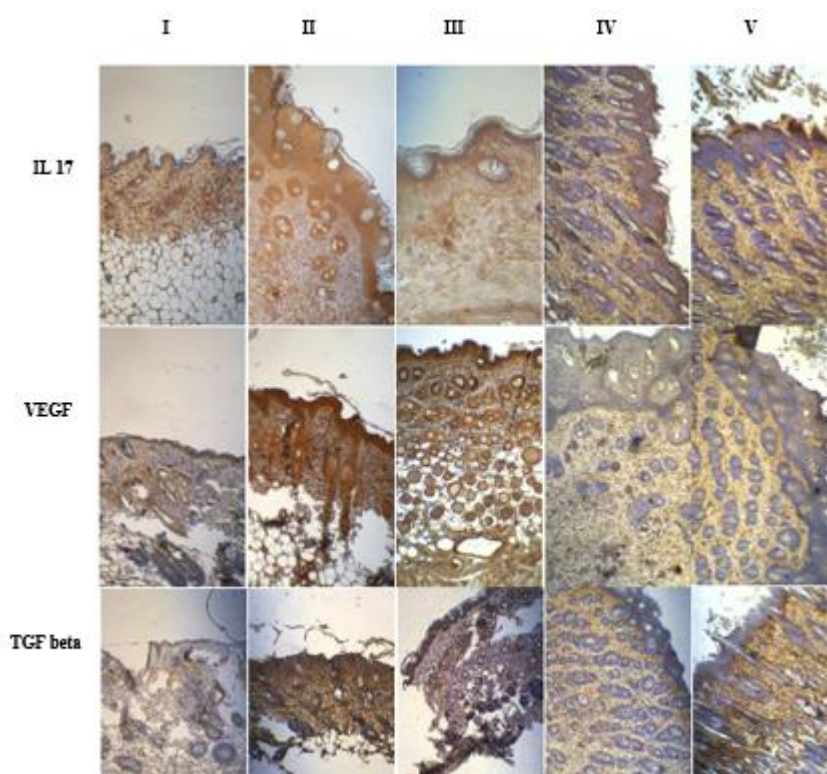


Figure 1: Immunohistochemistry of IL 17, VEGF, and TGF beta of mice skin groups. Group I: Healthy, group II: Psoriasis induction group, group III: Clobetasol group, group IV: Grape extract, and group V: Grape petroleum ether. Group II show more intense brown color indicate positive reactions for IL-17, VEGF, and TGF- beta. Less intensity in color of IL-17 is seen in groups III, IV, and V. Less intensity in color of VEGF is seen in group IV and V. Less intensity in color of TGF-beta is seen only in group III. Olympus microscope lens power 10. Skin samples taken on day five.

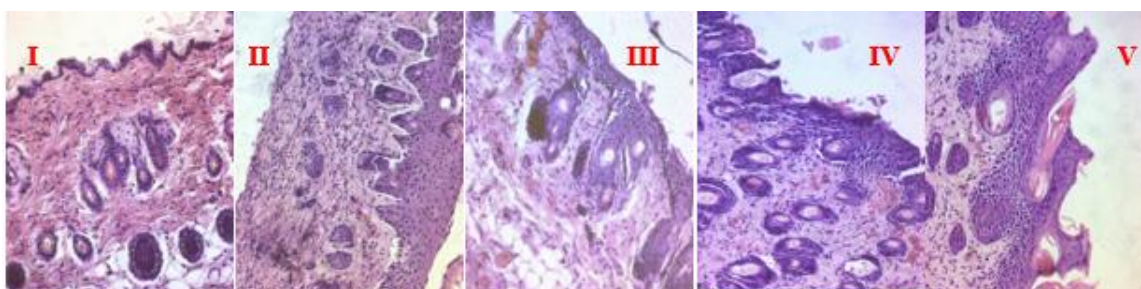


Figure 2: Histopathology sections of mice skin groups. Group I: Healthy, group II: Psoriasis induction group show all histopathology changes of psoriasis like munro abscess, hyperkeratosis, parakeratosis, lengthening of rete ridges, acanthosis, papillary papillae congestion, and dermis lymphocytic infiltrate. Group III: Clobetasol group, group IV: Grape extract, and group V: Grape petroleum ether. Groups III, IV, and V showed better munro abscess and less dermis lymphocytic infiltrate. Group IV and V showed improvement of parakeratosis. Group V showed decrease in lengthening of rete ridges. Stain of H&E of the back mice skin used. Olympus microscope lens power 10. Samples taken on day five.

Discussion

Previous studies were revealed that grape seed extract was known to be rich in proanthocyanidins which are potent anti-inflammatory agents that prevent oxidative stress and it was found that they are soothing psoriasis symptoms by inhibiting the inflammatory response and reducing oxidative damage²³.

Highly significant increase was found in IHC score of VEGF, and TGF-beta1 while significant increase in IL-17, A high significant increase in all of the histopathology parameters and significant increase in munro abscess was found between the healthy and psoriasis group. This indicate that all those are suitable parameters to be use for the evaluation of the grape as a treatment therapy for psoriasis. A previous study published in 2019 had been shown that imiquimod can induce psoriasis in mice were IL-17 target keratinocyte mainly and control infiltration of neutrophil¹³. Vascular endothelial growth Factor-A is originated from activated keratinocytes essentially in psoriasis skin patient²⁴. The microvascular psoriatic lesion proliferation is related to the effect of both VEGF-A and Ang-Tie2 system and the decrease in those mediators

may improve psoriasis symptoms of the lesion where they may play a major role in plaque psoriasis vascular proliferation^{25,26}. In relation to IL 17A, imiquimod increase it signaling by increase the epidermal IL17A expression as a result of mobilization of Th 17 cells. The imiquimod induced psoriasis method resemble several human features of inflammatory skin psoriasis²⁷.

More over when comparing the grape extract with psoriasis induced groups, it was found that there were a significant decrease in IL-17 and VEGF. A previous study stated that the psoriatic typical inflammatory marker VEGF inhibited by *Vitis vinifera*²⁸. Yang et al, in 2022 stated that proanthocyanidins which is found in grape, can decrease the concentration of proteins and their mRNAs of IL-17, VEGF and others. This indicates the anti-inflammatory effect of *Vitis vinifera*²³. Also one study use *Vitis vinifera* seed extract showed the anti-inflammatory role of the extract may be comparable to that of diclofenac sodium with less side effect²⁹. Moreover, Proanthocyanidins are natural extracts that have anti-inflammatory, immunomodulatory, anti-angiogenic and other effects³⁰, where proanthocyanidins inhibit endothelial migration of cells,

VEGF expression, and vascularization that made it with good anti-angiogenic properties³¹. One of the main compounds that present in *Vitis vinifera* is Resveratrol which consider as the main stilbene in grapes³². Both resveratrol stilbene and pterostilbene have a measured antioxidant activity in 1,1-diphenyl-2-picrylhydrazyl assay³³. Resveratrol have activated SIRT1³⁴ where the polymorphism of rs7069102 gene related to SIRT1 is one of the psoriasis early onset associations and is responsible for keratinocyte proliferation³⁵. Regarding the histopathology reading scores like munro abscess, parakeratosis, and dermis lymphocytic infiltrate when compared with psoriasis induction group were found significant. The histopathology study of proanthocyanidin experiment were found a decrease inflammatory cell infiltration in skin lesions²³.

The petroleum ether group, when compared with psoriasis group, it was found that the immunohistochemistry scores of IL-17 and VEGF were significantly decrease. This indicates better shift toward normal angiogenesis balance due to decrease VEGF. It had been previously being proven the vascular endothelial cell and epidermal keratinocytes are secreted growth factors and cytokines through upregulation of adhesion molecules signaling which induce inflammation of psoriasis⁴³. This led to disturbed the normal balance between the angiogenic mediators (VEGF, TGF- β -transforming growth factor, FGF2-fibroblast growth factor, and angiopoietin) with anti-angiogenic mediators (Thrombospondins and angiostatin)³⁷. Also, it showed in this petroleum ether fraction group, the histopathology scores of munro abscess, parakeratosis, lengthening of rete ridges, and dermis lymphocytic infiltrate were significantly decreased. This significant change gives an idea about the response to treatment against imiquimod psoriasis like effect. That to say, one of the typical pictures of psoriasis is altered epidermal differentiation where the parakeratosis shows as skin scaling and the

imiquimod compound will start the induction of keratinocyte proliferation increasing parakeratosis as showed by the change of nuclei position toward stratum corneum with other changes of plaque psoriasis²⁷.

When the clobetasol group compared to psoriasis induction group, significant decrease was found in IL 17 and TGF-beta1. The benefit of steroid for better psoriasis symptoms occurs partly due to inhibition of Th17 immune signaling and strong antioxidant effect of steroids with other mechanisms³⁸. Also, munro abscess, hyperkeratosis, and dermis lymphocytic infiltrate are significant decrease. A study stated that clobetasol treatment in mice can cause a decrease inflammatory cell infiltrate in the epidermis and dermis with thickening of stratum corneum³⁹. Regarding VEGF both treatment groups of grab extract and petroleum ether show better result than the clobetasol. This indicate better anti-angiogenic properties. Many histopathology measurements had no difference with the clobetasol group indicate good improvement of those measures like munro abscess. No difference was found between the two groups of grapes (group IV and V). When the all-treatment groups were compared, it was found a difference regarding VEGF and TGF -beta in addition to a difference in hyperkeratosis and dermis lymphocytic infiltration.

Conclusion

Grape seed extract (*Vitis vinifera*) and its petroleum ether fraction have anti-inflammatory and anti-angiogenesis effect via thier depressive effect on IL 17 and VEGF respectively in addition to other histopathological chaneges improvment of munro abscess and less dermis lymphocytic infiltration which may indicate a promise future therapy for psoriasis. More extra-research is needed with larger number and other species of animal to confirm the pharmacological action of grape seed.

Declaration of competing interest

No known personal or competing financial interest's completion.

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