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Modulated effect of *Ammi majus L.* on Psoriasis-Like Skin Inflammation Caused by Imiquimod *in Vivo*

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ABSTRACT

Psoriasis is a chronic skin disease associated with an immune disorder, with clear manifestations and an easy clinical diagnosis. The purpose of this experiment is to evaluate the effect of Ammi majus on psoriasis- like inflammation induced by imiquimod in male mice. 40 mice used, divided into 5 groups (8 mice per group). All groups received imiquimod for psoriasis induction except for the first two groups, during all study days. The other third, fourth, and fifth groups were induced and treated once daily with wool fat, Ammi majus, clobetasol propionate, respectively, from the seventh day until the end of the experiment. The result showed that Ammi majus has anti-psoriasis effect by significantly lowering the PASI score, attenuating IMQ-induced cutaneous changes and significantly lowering the level of interleukins in the serum compared to the induced group. We concluded that the effect of Ammi majus against Imiquimod-induced psoriasis is probably through a mechanistic pathway that includes anti-inflammatory and anti-proliferative effects, and could be developed for therapeutic purposes in dermatology, especially psoriasis.

Key words: Psoriasis, *Ammi majus*, Clobetasol propionate

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INTRODUCTION

Psoriasis is an autoimmune disorder characterized by changes in the components of the skin, such as infiltration of white blood cells and an increase in blood vessels in the dermis, resulting in localized inflammation with enlargement of the epidermis. They generally appear as undesirable local symptoms [1-4]. To date, the pathogenicity is not entirely clear [5], although extensive research continues to explore the underlying mechanisms involved. Imiquimod (IMQ) is an immune response adaptor that was firstly approved by the U.S Food and Drug Administration [6], it is used for the topical treatment of genital and perianal warts caused by human papillomavirus. Additionally, for the treatment of other virus-related skin abnormalities [7,8]. Animal models have been recruited to mimic skin conditions such as human psoriasis for many years [9,10]. In a mouse model, topical application of IMQ has been confirmed to cause psoriasis-like skin inflammation [11]. Whereas, these laboratory animals are considered a model that can allow the explain the basic mechanisms and the estimate of proposed new treatments for psoriasis [12]. Humans have used plants as medicines for thousands of years, and there are many medicinal plants that can prevent diseases and reduce their complications as much as possible, because they are highly effective with fewer side effects [13,14]. *Ammi majus L.* (AM) is belongs to the Apiaceae plants, native to northern Africa, southern Europe, western Asia and India [15,16]. In Iraq, it is found in fields and gardens in several areas especially in Baghdad [17]. Several studies have evaluated the efficacy of *Ammi majus* in curing some skin disorders in vivo, so the aim of the study was to evaluate the efficacy of *A. majus* seed extract against psoriasis-like skin inflammation caused by Imiquimod in a mouse model.

MATERIALS AND METHODS

Drugs

Imiquimod)IMQ) was obtained commercially from Aldara TM 5% Cream. Clobetasol propionate 0.05% (DermovateTM Cream) was punched from a private pharmacy.

Preparation of plant extract

The seeds of the medicinal herb *Ammi majus* were purchased from Erbil, northern Iraq. The plant was then kindly cleaned to remove dirt and dried in the laboratory at room temperature for a few days. Then, the dry plant material was crushed followed by sieving to get a tough grind. The plant matter powder was then extracted with petroleum ether (40-60°C) by re-uX using a sohxlet for 10

h, and the resulting crude extraction solution was evaporated by a rotary vacuum evaporator and concentrated to obtain the fractional petroleum ether *A. majus* extract. The resulting crude extract was stored at 4 °C before formulation.

Preparation of a topical emulsion (water in oil)

Wool fat (70 g) was melted in a water bath at 75°C until it became a liquid. *A. majus* fraction extract (5 g) was added to a laboratory beaker containing up to 30 ml of distilled water and stirred until solutions were obtained. Then the last solutions were added to the melted wool fat baker and the final mixture was cooled with stirring until it was congeal .

Animals and study design

In this experiment, (40) adult male mice weighing (22-42 g), and their ages ranged between (6-7) weeks, were used. They were obtained from the animal centers of the University of Baghdad/Iraq after the study protocol was approved by the Iraqi Review Board (IBR) for the College of Pharmacy. The animals were housed in polypropylene cages and in environmentally controlled conditions in terms of humidity, temperature and appropriate lighting, with free access to water and diet. Laboratory mice were separated into 5 groups (8 animals in each group) and divided as shown in Table 1.

Table 1: Experimental groups and dose treatments.

Groups	Experimental Model
CON	Vehicle of *A. majus* ointment (wool fat) was applied to the mice at a dose of 62.5 mg/cm2 on their shaved backs and at a dose of 31.25 mg to their right ear, twice daily for 14 consecutive days.
AM	*A. majus* ointment was administered to mice at 62.5 mg on their shaved backs and at a dose of 31.25 mg in their right ears, twice daily for 14 consecutive days.
CON-IMQ	Mice were topically induced with imiquimod at 62.5 mg on their shaved backs and 31.25 mg on their right ears in a single dose daily for 14 consecutive days. 2 hours later, vehicle of *A. majus* ointment was applied to them in the same amounts, but twice daily, from the 7th to the last 14th day of the experiment.
AM-IMQ	Mice were treated with imiquimod at 62.5 mg on their shaved backs and 31.25 mg on their right ears in a single dose daily for 14 consecutive days. 2 hours later, *A. majus* ointment was applied to them in the same amounts, but twice daily, from the 7th to the last 14th day of the experiment.
CP-IMQ	Mice were received imiquimod at 62.5 mg on their shaved backs and 31.25 mg on their right ears in a single dose daily for 14 consecutive days. 2 hours later, clobetasol propionate ointment was applied to them in the same amounts, but twice daily, from the 7th to the last 14th day of the experiment.

Ear and skin thickness measurement

Ear thickness (mm) was measured for each rat using a digital micrometer, on days 2, 4, 6, 8 and 14 of the experiment. The change in ear thickness was used to demonstrate the gravity of inflammation. Also, the skin thickness of the dorsal region of each mouse (mm) was measured at the end of the experiment (day 14) to compare the thickness measured with that of the other groups.

Psoriasis area and severity index

This index PASI was adopted to assess the psoriasis score, which combines assessment of the severity of lesions and the extent of the affected area into a single indicator.

Inflammatory markers

Blood was taken through a cardiac puncture and serum was separated and stored at -20°C until analysis for determination of IL-23 (pg/ml) and IL-17 (pg/ml) levels.

Statistical analysis

Data were summarized by SPSS software. Using mean ± standard deviation, as well as one-way analysis of variance (ANOVA).

P values ≤ 0.05 were considered statistically significant.

RESULTS

Results for the PASI score, significantly at P ≤ 0.05, the data indicated an increase in the CON-IMQ group compared to the CON group.

Also, in the AM-IMQ group, topical application of *A. majus* caused a respectable decrease compared to the CON-IMQ group. In the CP-IMQ group, the score was diminished compared to the CON-IMQ.

Whereas in *A. majus* ointment only (AM) there was no observed change in the score during the study (Table 2).

Table 2: Comparison of psoriasis area score and severity (PASI) among study groups.

Groups	PASI Score
CON	Zero
AM	Zero#

CON-IMQ	7.06 ± 1.11*
AM-IMQ	3.75 ± .48*#
CP-IMQ	2.41 ± .33*#

Each value represents mean ± standard deviation (SD). * Significantly different with respect to the CON group. # P ≤ 0.05 significant in comparison with the CON-IMQ group.

Moreover, there was a significant increase in both ear and dorsal skin thicknesses in the CON-IMQ compared to that in the CON group. But in the AM-IMQ group, the measured ear and dorsal skin thicknesses were clearly decreased compared to the CON-IMQ group. While in the CP-IMQ group, there was a significant decrease in the thickness of both ear and back skin as compared to the CON-IMQ as shown in Tables 3 and 4.

Table 3: Comparisons of ear thickness (mm) among study groups.

Duration (Day)	CON	AM	CON-IMQ	AM-IMQ	CP-IMQ
2	0.22 ± 0.011	0.23 ± 0.014#	0.27 ± 0.011*	0.23 ± 0.016#	0.22 ± 0.013#
4	0.23 ± 0.011	0.23 ± 0.007#	0.320 ± 0.013*	0.23 ± 0.019#	0.23 ± 0.013#
6	0.23 ± 0.010	0.24 ± 0.008#	0.36 ± 0.011*	0.24 ± 0.022#	0.24 ± 0.015#
8	0.23 ± 0.008	0.24 ± 0.008#	0.41 ± 0.010*	0.26 ± 0.028#	0.25 ± 0.018#
14	0.22 ± 0.011	0.23 ± 0.014#	0.27 ± 0.011*	0.23 ± 0.016#	0.22 ± 0.013#

Each value represents mean ± standard deviation (SD). * Significantly different when compared with the CON mice. # P-value ≤ 0.05 significant in comparison with CON-IMQ group.

Table 4 : Comparisons of dorsal skin thickness (mm) among study groups.

Groups	Skin thickness (mm)
CON	0.3725 ± 0.06756
AM	0.3863 ± 0.02066
CON-IMQ	0.6063 ± 0.04307
AM-IMQ	0.4225 ± 0.03370
CP-IMQ	0.4013 ± 0.04643

ANOVA /Skin thickness

Test - groups	Sum - Squares	df	Mean- Square	F	Sig0.
Between- groups	0.295	4	0.074	36.367	0
Within -groups	0.071	35	0.002		
Total	0.366	39			

Each value represents mean ± standard deviation (SD). * Significantly different when compared with the CON mice. # P-value ≤ 0.05 significant in comparison with CON-IMQ group.

As for the results related to interleukins, there was a high increase in both serum levels of IL-23 and IL-17 in the CON-IMQ compared to the corresponding levels in the CON group. In the CP-IMQ, there was a marked decrease in IL-23 and IL-17 serum levels compared to the CON-IMQ, and by comparing AM-IMQ with the CON-IMQ group, there was an observed decrease in IL-23 and IL-17 serum levels. Finally, serum IL-23 and IL-17 levels were significantly increased in the AM group compared to the CON-IMQ (Figure 1).

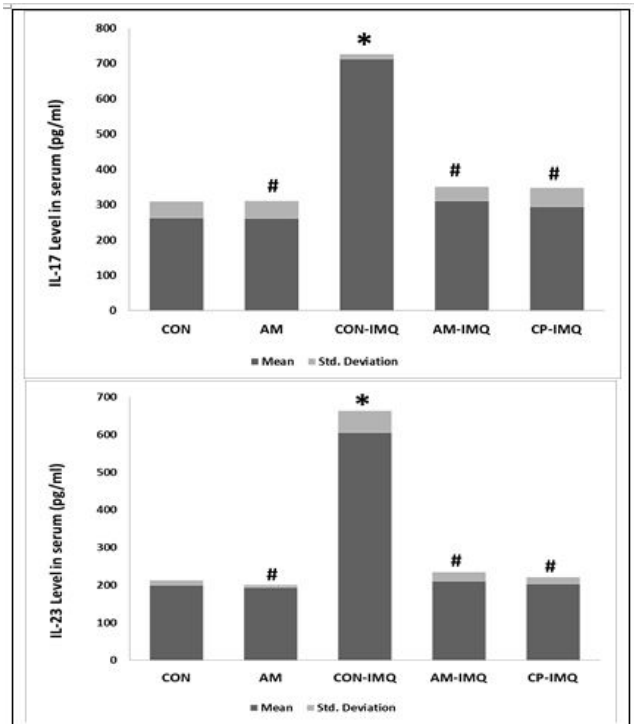

Figure 1: The comparison of mean: (a) IL-17 and IL-23 levels among study groups .* Significantly different when compared with CON mice. # P-value ≤ 0.05 significant in comparison with CON-IMQ group.

DISCUSSION

Based on the evidence of many previous studies, the imiquimod-inducible psoriasis mouse model summarizes signs of inflammatory skin disorders like to human psoriasis. Like the thickness of the epidermal layer, prolongation of retinal-like protrusions and hyper-keratosis next imiquimod induction [18-20]. Physicochemical properties of IMQ can explain the kinetics of penetration and retention of skin layers. The low molecular weight allows easy penetration into the skin but due to its lipophilic the molecule is retained primarily in the stratum corneum [21]. Skin changes appeared from the second day, as the overall result of PASI came to highlight the severity of psoriasis and in particular by noticing the crusting that represents the formation of the scale. The stratum corneum layers are separated thus promoting better IMQ skin penetration [22]. Significant skin thickening was observed on both the back and the treated ear starting from the second day and it increased throughout the study period. Systemic toxicity was also demonstrated synchronous with the appearance of localized psoriasis signs. In skin disorders arising from inflammation such as psoriasis, different types of cytokines such as interleukins are observed to be involved in modulating inflammatory responses [23]. IL-17 has a decisive role in the pathogenicity of psoriasis as its cutaneous expression is tight associated with the up growth of skin lesions, with an increase starting one day after IMQ treatment [24]. *A. majus* contains four main linear furocoumarins, the most pharmacological of which is xanthotoxin [25]. Previous studies have proven the effectiveness of xanthotoxin in the treatment of psoriasis, and this is consistent with what we have found in this study [26,27]. *A. majus* has also been shown anti-inflammatory activities due to its richness in phytochemicals [28]. For its dermatological role, Shinde and Mohite, have conducted studies on the composition of an herbal gel containing *Aloe Vera L* and Ammi Majus L for use topically on the skin [29-31].

CONCLUSION

When *Ammi majus L* seed extract was used against psoriasis-like inflammation induced by Imiquimod in male mice and compared to clobetasol propionate, modulation of skin thickness changes and inflammation parameters was observed.

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