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Original Article

Pharmaceutical Characterization and *in vivo* Evaluation of the Possible Anti-Inflammatory Effect of Topical Allopurinol Gel in an Animal Model

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Abstract

Dermatitis, like inflammation, is a group of common dermatological symptoms and may be associated with systemic and skin diseases. The objective of the current study is to evaluate the potential anti-inflammatory effect of the topical allopurinol against inflammation like skin dermatitis induced by1-isobutyl-1H-imidazo [4,5-c]quinolin-4-amine (IQA) in mice model. The current study allocated the thirty-two mice into four groups (n=8) as follows: i) control group, mice where a white petroleum jelly base applied topically on the dorsal of mice once daily; ii) induction group, mice were received IQA cream (62.5 mg) of (5%) on their back once daily; and iii) the treatment group, mice were treated with both (62.5 mg) of (5%) IQA and (5%) allopurinol gel topically; the betamethasone group, mice were treated with both (62.5 mg) of (5%) IQA and betamethasone ointment topically. All groups were treated daily for seven days period. The allopurinol-treated group exerted non-significant differences compared with the induction group in both visional and histopathological changes. The present study revealed that the allopurinolgel (5%) did not affect skin inflammation- induced by IQA in the laboratory mice.

Keywords: Allopurinol, IQA, Inflammation, Dermatitis

1. Introduction

Dermatitis, like inflammation, is a painful and disfiguring lesion characterized by red and swollen skin. The more significant impact is that the disease is curable by topical corticosteroids, but it may affect the quality of life (1). Skin lesions are mostly sharply demarcated red papules and plagues covered by scale mostly noticed on the extensor surface and scalp due to inflammation processes including infiltration of neutrophils and production of proinflammatory factors. Morphological variations of the lesion are common between individuals; this is determined by genetic and environmental influences (2).

Dermatitis lesions may affect children under five years; the worldwide prevalence of dermatitis is increasing, with about 70% of cases happening before the age of five (3).

The prevalence of dermatitis has increased remarkably in industrialized countries over the past four decades, currently approaching 15-30% of children and 2-10% of adults.1, 2 Although both endogenous and exogenous factors are associated with AD pathogenesis, accumulating evidence suggests that T helper (Th) 2 cytokines are pivotal effector molecules

for the development of AD (4).

Although the etiology of dermatitis-like inflammation remains unknown, some evidence brought the light the role of genetic predisposition in concomitant with other factors, including environmental factors like streptococcal infection, exposure to certain medications like antimalarial drugs and TNF-α inhibitors, and other factors may include smoking and alcohol misuse (5-7).

Allopurinol action inhibits xanthine oxidase, which is responsible for the successive oxidation of hypoxanthine and xanthine with the resultant production of uric acid (8). In addition, it stimulates the action of hypoxanthine-guanine phosphoribosyl transferase (9). Interleukin-8 (IL-8) is considered one of the critical mediators of the inflammatory process. It is a chemoattractant and a potent angiogenic factor (10).

This study aims to explore whether topical application of allopurinol has a role in the remission of the dermatic lesion in mice models.

2. Materials and Methods

2.1. Preparation of Allopurinol Topical Gel

As a moistening agent, the gel was prepared by dissolving allopurinol (5% w/w) in glycerin (40% w/w). The mixture was added to hydroxyl propyl methyl cellulose (HPMC). The latter was prepared by dispersing the calculated amount of polymer in warm water with constant stirring using a magnetic stirrer at a moderate speed. However, the prepared gel was stored in a wide-mouth glass jar covered with aluminum foil and a plastic lid and kept in a dark and cold place (11).

2.2. Physicochemical Evaluation of Prepared Allopurinol Gel

2.2.1. Visual Examination

The gel was examined for its physical properties by visual inspection of color, clarity, homogeneity, and phase separation (12).

2.2.2. PH Determination

The pH meter was used to measure the pH of the gel formulation. Five grams (5 gm) were dissolved in deionized water to obtain aqueous gel solutions at room temperature. The pH standards (4 and 10) were used to calibrate the pH meter before use (13).

2.2.3. Spreadability

The spreadability is an important test in the evaluation of the behavior of the prepared gel. Spread ability was carried out by using a glass plate (20×20). A circle was drowning on the plate then (0.5) g of gel was placed inside the circle. Another one sandwiched the gel. A weight of 500 mg was placed upon the upper plate for 5 min. The excess gel was scrapped off carefully after removing the weight. A weight of 20 g was fixed to the upper plate in such a way that allows the slip of the upper plate only by the force of the weight tied to it. The time taken for the upper plate to move a distance of 6 cm was recorded. The process was repeated thrice, and an average of three records was recorded (14).

2.3. Animal Experiments

Animales of the study were male adult albino mice weighing (25-32 g); they were obtained from the (national center for drug control and research) (NCDCR) in Iraq. Mice were fed on a standard pellet diet and water *ad libitum*, and the room was under controlled conditions (12 h light-dark cycle at 22±2 °C). Animals were acclimatized for 7 days before starting the experiments.

2.4. Mice Models of IQA- Induced Inflammation Like Dermatitis

The study model included 32 mice, divided into three groups (n=8). The backs of the mice of all groups were shaved for topical application. These groups were divided as follows: firstly, the control group: mice, received a petrolatum base once daily for seven days. Secondly, aninduction group was treated with (62.5 mg) of (5%) IQAcream daily for seven days.The allopurinol group was treated with both (62.5 mg) of (5%) IQA cream and (5%) allopurinol gel once daily for seven days. The betamethasone group was treated with both (62.5 mg) of (5%) IQA cream and betamethasone ointment once daily for seven days.

2.5. Preparation of Tissue Homogenate

One gram of freshly harvested tissue was stored in 9 ml phosphate buffer saline. Mortor and pestle were

used for tissue homogenization. The tissue was centrifuged by cold centrifuge at 5000 rpm for 10 minutes. The supernatant was stored at (−80 °C) for further tests (15).

2.6. Evaluation of Gel Characterization

This may include physiochemical properties such as clarity, color, homogeneity, and syneresis, as well as pH, spreadability, and irritation tests.

2.7. Measurement of Inflammation Like Dermatitis Biomarkers

2.7.1. Estimation of Interleukin 8(IL-8) by ELISA

Estimation of mouse IL-8 was accomplished by ELISA test, which is used for *in vitro* determination of the mouse IL-8 level in the serum.

The principle of this test is a sandwich ELISA test in which the plate was pre-coated with mouse IL-8 monoclonal antibody. The detecting kit consists of a biotin-labeled polyclonal antibody. The reaction was carried out by adding samples and biotinylated antibodies to the wells and washing them out with phosphate-buffered saline (PBS), followed by adding Avidine-peroxidase conjugates to wells of ELISA plate, reactant thoroughly washed out, then tetramethylbenzidine (TMB) substrate was used as a coloring agent. By reaction with peroxidase, TMB turns blue and finally is yellow by the action of acid. The following steps were followed according to the manufacturer's instructions regarding procedure and determination of the positive and negative results (16).

2.7.2. Scoring of Inflammation Intensity

The intensity of back skin inflammation depended on the erythema and thickness; the overall scoring did not include the affected skin area in the mouse model.

The scale 0-4 was used to score the parameters (erythema, scaling, and thickness). Zero means none; one means slight; two means moderate; three means marked, and four means very marked. The erythema level was carried out depending on the red taint scoring table. The cumulative erythema score, scaling, and degree of thickness indicated the severity of inflammation (17).

2.8. Statistical Analysis

Statistical Package for Social Sciences (SPSS version 22) was used for statistical analysis. Descriptive statistics compared numerical data and formulated as mean and standard deviation (SD). Mann-Whitney U test was used to compare the study's two histologically scored groups. Scoring of histopathological changes were performed by semi-quantitative analysis in which grade (0- 4) was given for each sample according to changes. The ANOVA and post-Hock test samples were used to compare the study groups, and the ($P \leq 0.05$) was considered significant.

3. Results

3.1. The Physiochemical Properties of Allopurinol Gel

The allopurinol gel appearance was much clear and transparent. The developed gel

formulae showed high homogenization with no lumps and syneresis

(Table 1).

Table 1. Composition of topical gel formulations of allopurinol 5% (w/w)

Formula Ingredient	Fraction
Allopurinol powder	5 g
HPMC powder	4 g
Glycerin liquid	4 g
Water up to	100 ml

3.1.2. PH Determination

The pH of the allopurinol gel was about 5.3. This level was deemed appropriate to reduce the risk of skin irritation during the usage of the formula. Then the process is repeated thrice to be expressed as the mean of pH with standard deviation (Table 2).

Table 2. pH determination

Medications	Concentration (W/W)	Mean±Standard Deviation
Allopurinol gel	5%	5.328±0.5

3.1.3. Spreadability

The spreadability values indicate that the gel of allopurinol was quickly spreadable by a small amount

of shear. The spreadability of formulated gel (allopurinol) was 7.8 cm/sec. Hence spreadability of the formulation was good because of its high micro-viscosity; it has a higher spreadability than other formulations (Table 3).

Table 3. Spreadability

Topical formulae	Spreadability (cm/sec)
Allopurinol gel (5%)	7.8±2.5

3.1.4. Irritation Test

The skin irritation test is related to the evaluation of the created gel, which confirmed the absence of any irritation on the treated surface by application of allopurinol gel on volunteers' skin after approval of the ethical consent of the participants.

3.2. Scoring for Visual Parameters of Dermatic Disease

3.2.1. Scoring of Erythema

The control group treated with white petroleum jelly showed non-erythematous skin, while IQA groups exerted very severe erythema (*P*-value<0.05) whatever allopurinol showed no considerable difference compared to the IQA group (*P*-value>0.05) as shown in figure 1.

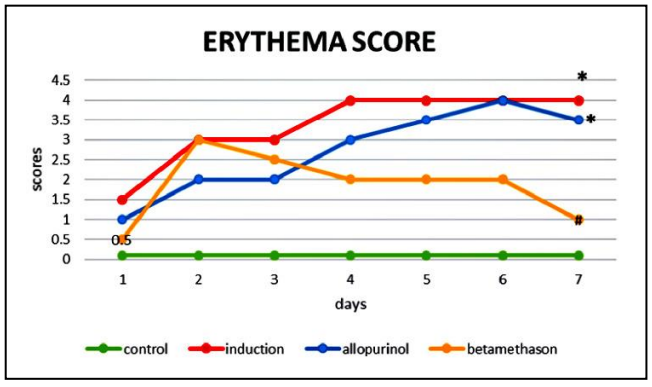

Figure 1. The effects of IQA and allopurinol on erythema visual score have been shown a statistical significant differences have been seen clearly in the induction group compared to the control group.* means significant differences compared to control at (*P*<0.05); #non means significant differences compared to control at (*P*<0.05)

3.2.2. Scoring of Scaling

IQA group exerted a very severe scaling compared to the control group treated with white petroleum jelly only (*P*-value<0.05). Mann-Whitney *t*-tests found no significant differences in mean scaling imiquimod and allopurinol treated groups with *P*-value>0.05, as mentioned in figure 2.

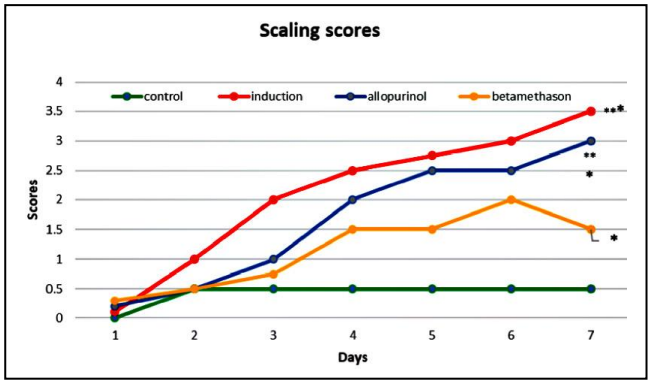

Figure 2. The effects of IQA and allopurinol on scaling scores have shown significant changes compared to the control group. *means significant differences compared to control at (*P*<0.05); **significant changes in compared to betamethasone group

3.3. The Effect of Allopurinol on Serum Interleukin 8 (IL-8) Levels

This set of results showed the impact of allopurinol on IL8 in IQA-induced dermatitis-like inflammation in the mice model. The average level of serum IL-8 in the IQA group was significantly higher than that of the control (Figure 3) IQA (0.073±0.008 pg./g) emphasize versus control (0.012±0.0042 pg./g); *P*≤0.05). On average, the allopurinol group was shown no significant reduction in IL-8 in comparison to the IQA group, IQA group (0.073±0.008 pg./g) versus the allopurinol group (0.069±0.006 pg./g); *P*-value>0.05) (Figure 3).

3.4. Histopathological and Histomorphometric Study

3.4.1. The Effects of Allopurinol on Epidermal Thickness

Skin sections of the control group revealed skin with a normal appearance (Figure 4), in which there is the normal epidermal thickness and normal adnexa (Figure 4A). The average epidermal thickness was about (1.7±0.02) micrometer (Figure 4B).

In the induction group, the skin sections showed extensive epidermal thickening with marked extensions of the rate pegs into the dermal layer (Figures 5A and 5B). The average epidermal thickness was (3.2±0.02) micrometer (Figure 5C). The skin epidermis in the treated group showed moderate thickness (Figure 6A). The average epidermal thickness was (2.9±0.02) (Figure 6B). Histomorphometric measurements of the control, induction, and treated groups are shown in table 4.

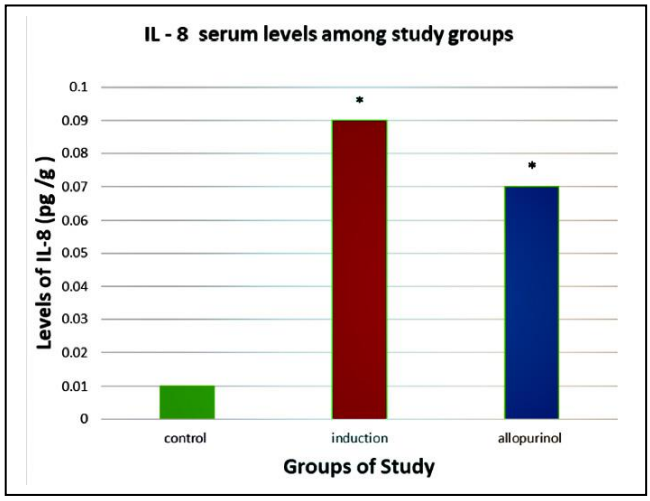

Figure 3. the level of IL-8 (pg./g) in skin homogenate of study groups, the induction group showed an elevated level of IL-8 in comparison to the control group, while the allopurinol treated group non significantly reduced the level of allopurinol in comparison with the control group. *means significant differences compared to control at (P<0.05). *means significant differences compared to control at (P<0.05)

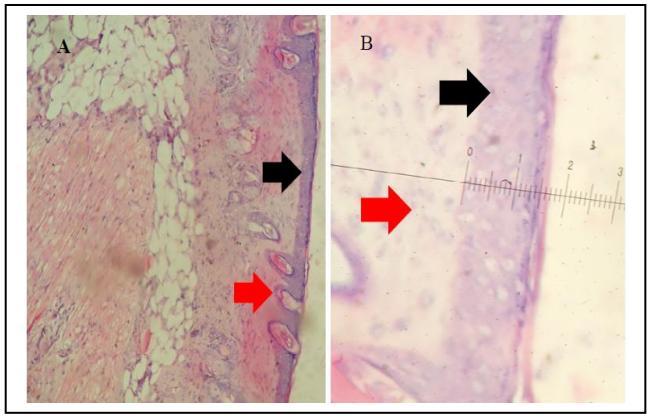

Figure 4. A skin section from animals of the control group reveals a normal epidermal layer (black arrow) and dermal layer (red arrow) **A)** 125X **B)** 500× H&E

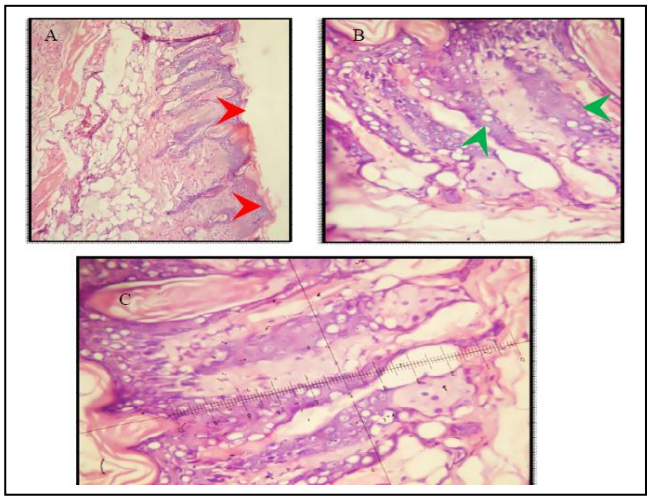

Figure 5. Skin section from induction group shows epidermal thickening (red arrow) with extension of rete pegs (green arrow) to produce Psoriasis like model **A)** 125X **B)** 500X H&E. **C)** Skin section from induction group shows the histometric measurement of the epidermal extension in induction group 500× H&E

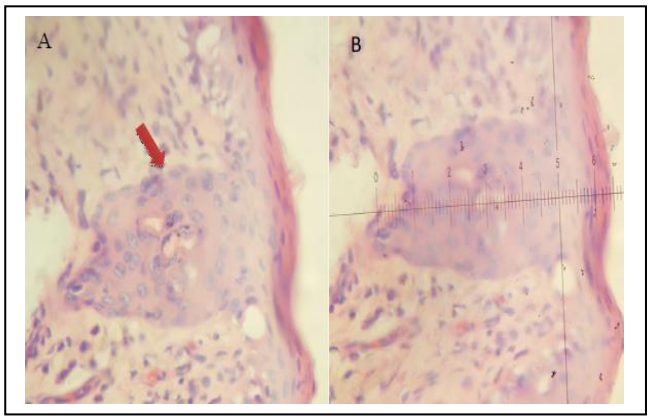

Figure 6. A) Skin section from allopurinol treated group shows epidermal thickening with an extension of rete pegs (red arrow), **B)** histometric measurement of the epidermis **A)** 500X **B)** 500× H&E

Table 4. The Effects IQA and allopurinol on dermal thickness

Groups	Epidermal thickness (micrometer)
Control	1.7±0.02
Induction (IGA)	3.2±0.02
Allopurinol	2.9±0.02

3.4.2. The Effects of Allopurinol on the Spleen

Histopathological study of the spleen in the study groups revealed that in the control group, the spleen shows a normal appearance, with normal red and white pulp (Figure 7). The induction group showed marked deposition of amyloid-like material in the white pulp with congestion of the red pulp (Figures 8A and 8B). The treated group showed mild deposition of amyloid-like material in the white pulp (Figure 9) and hemosiderosis in the red pulp.

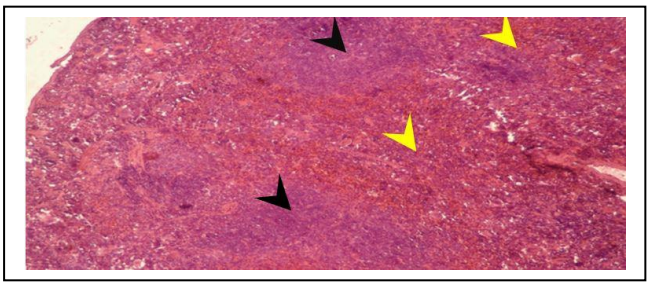

Figure 7. Section of spleen from control group revealed normal white pulp (black arrow) and red pulp (yellow arrow) 500× H&E

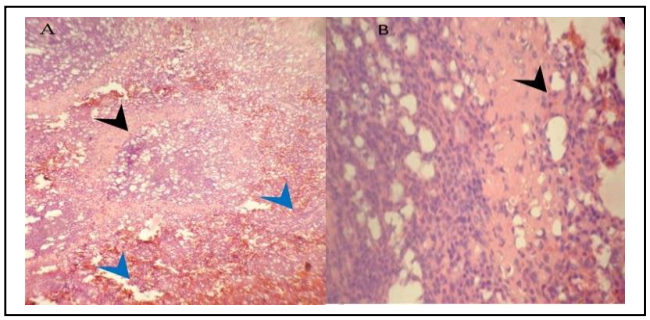

Figure 8. Splenic section from induction group shows deposition of amyloid-like material (black arrow) around the white pulp, congestion of the red pulp (blue arrow) **A)** 125X, **B)** 500× H&E

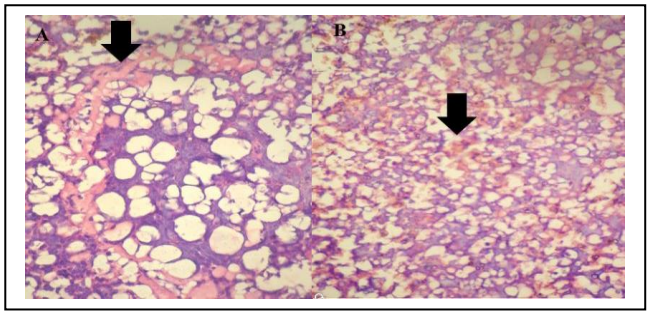

Figure 9. Splenic section from allopurinol treated group shows deposition of amyloid-like material (black arrow A) around the white pulp in addition to hemosiderin deposition (black arrow). 500× H&E

4. Discussion

This study searched for the anti-inflammatory and anti-psoriasis possibility of allopurinol as a new area for allopurinol effects. The properties of allopurinol gel and its characterization is promising for the availability of a novel dosage form that can be used for topical purposes (11, 18). Allopurinol gel is prepared by the traditional method with acceptable physiochemical properties suitable for topical application and its lack of irritating effect (19). The skin inflammation induced by IQA is represented by visual erythema and scaling and irritation that is noticed clearly on the dorsal skin of mice and recording by the measured scores model (11). Also, IQA mediated its effects by elevating the level of proinflammatory factors such as IL-17 and IL-23 besides IL-8 angiogenetic factors. This high level of IL-8 had been shown in IQA, and this result is compatible with other previous studies (20, 21).

Furthermore, Chuo, Tung (22) showed a strong association between the level of serum IL-8 and inflammation-like dermatitis. The systemic effects of IQA displayed histological changes in spleen tissues by deposition of amyloid-like structures, which is agreeable with another study (23). The allopurinol showed less or no anti-inflammatory effects at histopathological levels or biomarkers levels, as well as the visual features of the skin. Although, the topical application of allopurinol showed a reversing radiation-inducing dermatitis in an animal model (24). Also, it had a role in regenerating damaged skin and wound healing due to epidermal drug retention (11). While allopurinol gel showed no or less effect against inflammation like skin dermatitis, that may be attributed to less penetration ability of allopurinol gel attributed to its water solubility (18) or required concentration of more than 5% to reversing the IQA induced inflammation like dermatitis.

The researchers could be concluded that allopurinol-HPMC (5%) gel had no statistically significant difference in terms of visual and histopathological effects on IQA-induced inflammation like dermatitis in the mice model.

Authors' Contribution

Study concept and design: M. R. A.
Acquisition of data: H. F. A.
Analysis and interpretation of data: M. A. R. and H. F. A.
Drafting of the manuscript: W. K. Y. A.
Critical revision of the manuscript for important intellectual content: M. R. A.
Statistical analysis: H. F. A.
Administrative, technical, and material support: H. F. A.

Ethics

The Bioethical Committee approved a procedure in this study of Medical College -University of Misan (No. 100 in 9\4\2021).

Conflict of Interest

The authors declare that they have no conflict of interest.

Acknowledgment

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