Crocin ameliorated skin tissue inflammation in atopic dermatitis in mice

Abdullah Alyoussef*

ABSTRACT

Background: Atopic dermatitis (AD) is considered a chronic recurrent inflammatory skin disease. In addition, crocin is the major carotenoid compound found in Gardenia jasminoides. It is previously proved to produce anti-inflammatory actions. Therefore, we conducted this research to investigate the therapeutic effects of crocin on a mice model of AD.

Methods: Mice were investigated for the number of scratches and dermatitis score. Skin was isolated and used for measurements of gene and protein expression of β-catenin, NFκB, TNF-α and IL-1β.

Results: Authors found that crocin significantly reduced the number of scratches, ear thickness and dermatitis score. In addition, crocin ameliorated AD-induced elevation in the expression of β-catenin, NFκB, TNF-α and IL-1β.

Conclusions: Crocin ameliorated DNCB-induced AD in mice via blockage of β-catenin with subsequent reduction in inflammatory pathway.

Keywords: β-catenin, Interleukin -1β, Nuclear factor κB, Tumor necrosis factor

INTRODUCTION

With rising progressing in the last decades, atopic dermatitis (AD), or as well called atopic eczema, is a widespread chronic and retrograde inflammatory skin disease. AD has become a universal health concern, especially in developed countries associated with impairment of the quality of life. Considered the superior cause of non-fatal condition in the skin, AD is estimated to influence about 230 million worldwide according to WHO.

Therapeutic objectives focused mainly of attenuating pruritus as well as controlling the disease spread. However, the approved treatment options are limited. Therefore, AD affects patients and their families both psychosocially and financially. Therapeutic strategies of AD depends on application of local or systemic corticosteroids with unfavorable side effects Therefore, there is great demand to find new therapeutic agents.

Crocin is carotenoid compound and major component of Gardenia jasminoides. It is responsible for the color of saffron. Crocin has many effects as analgesic, antipyretic, antioxidant, anti-inflammatory and anticancer. Although many studies were conducted for investigation of anti-inflammatory effects of crocin, the therapeutic effects of crocin on attenuating skin inflammation in AD was not fully studied. Therefore, authors conducted this study to determine effect of crocin on β-catenin/NFκB pathways in AD.
METHODS

Animals

The local ethical committee approved animal protocol. Four-week-old BALB/c mice were distributed into four groups, each group consisted of ten mice. The first two groups were kept as control and one of them was treated with 20 mg/kg crocin (Sigma Aldrich Chemicals Co., MO, USA) subcutaneously three times weekly for three weeks. In the other two groups, AD was induced after 24 hours of dorsal hair removal by 100 µl 1% 2,4-Dinitrochlorobenzene (DNCB) in acetone: olive oil (3:1) on back skin. After five days, 150 µl 0.2% DNCB was applied three times weekly for three weeks. A group of rats was treated with 20 mg/kg crocin subcutaneously three times weekly for three weeks.

Evaluation of ear thickness

The thickness of ears was measured in a weekly basis using a micrometer.

Table 1: The primer sets used.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Accession number</th>
<th>Sequence (sense, antisense)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α</td>
<td>X02611</td>
<td>5’-TACTGAACCTCCGGTGATTGTC-3’ 5’-CAGCCTTGCCTCTTGAGAACC-3’</td>
</tr>
<tr>
<td>IL-1β</td>
<td>NM_008361</td>
<td>5’- AGTTGACGACCCCCAAAGAT-3’ 5’- GACACGCCCCAGTCAAGG-3’</td>
</tr>
<tr>
<td>NFκB</td>
<td>NM_008689</td>
<td>5’-GAAATTCCTGATCCGACAAAAC-3’ 5’-ATCACTTCAATGCGCTGTGTA-3’</td>
</tr>
<tr>
<td>GAPDH</td>
<td>M32599</td>
<td>5’- ACCACAGTCCTCATC-3’ 5’- CACCACCTGTTGCTAGCC-3’</td>
</tr>
</tbody>
</table>

Number of scratches

On the last two days before sacrifice, the number of scratches of each mouse were counted for 10 minutes for five times per each mouse. Scratches were defined as the movement using the hind paws.

Determination of dermatitis score

Mice were given; scores 0 (none), 1 (mild), 2 (moderate), and 3 (severe) for the following signs of atopic dermatitis edema, erythema/hemorrhage, scaling/dryness and excoriation/erosion. Sum of all scores was used as dermatitis score.

ELISA determination

Commercially ELISA kits were used for TNF-α, IL-1β and β-catenin (Thermo Fisher Scientific Inc., Waltham, MA, USA).

RT-PCR

Standard methods and kits were used for NFκB, TNF-α and IL-1β mRNA levels using specific primers as described previously by group 6 (Table 1).

Statistical analysis

Comparison was evaluated by ANOVA followed by post hoc Bonferroni correction. SPSS version 20 was used. Statistical significance was defined as p<0.05.

RESULTS

Crocin ameliorated AD-induced elevation in β-catenin expression

AD elevated protein expression of β-catenin mice skin by 3.18-fold when compared with control group. Treatment of mice with crocin blocked β-catenin expression in AD group without affecting the control group (Figure 1).

*significant difference compared with the control groups at p<0.05.

Figure 1: Effect of AD alone and in combination with 20 mg/kg crocin on skin levels of β-catenin.
Crocin attenuated AD symptoms in mice

AD mice displayed severe erythema, erosion and dryness in dorsal skins. In AD mice, authors found number of scratches (15.9±1.4 scratches per 10 minutes), dermatitis score (5.7±0.48) and ear thickness (0.27±0.02 mm) compared with control mice (0.94±0.09 scratches/10 minutes, 5.7±0.48 and 0.03±0.004 mm, respectively). However, crocin ameliorated number of scratches (5.97±0.52 scratches/10 minutes), dermatitis score (2.9±0.26) and ear thickness (0.13±0.012 mm) without affecting control group (Figure 2).

Crocin blocked AD-induced elevation in the inflammatory pathway

Analysis of inflammatory pathway in mice skin revealed significantly increased gene expression of NFκB, TNF-α and IL-1β by 4.97-, 3.94- and 3.24-fold, respectively and increased skin levels of TNF-α and IL-1β by 2.65- and 2.97-fold, respectively in AD mice compared with control mice. Crocin blocked all these effects (Figures 3 and 4).

DISCUSSION

Crocin was used previously in two studies in treating AD via histamine inhibition 7 or blocking of ERK-MAPK/NFκB/STAT1.8 However, authors found that crocin ameliorated AD symptoms associated with reduced expression of β-catenin in AD group. β-catenin pathway controls a wide variety of processes as cell adhesion, differentiation and inflammation, which are the whole marks of atopic dermatitis. Blocking β-catenin pathway showed significant roles in some dermatological diseases such as diabetic cutaneous ulcers, radiation-induced damage and wound healing. However, no previous study illustrated the role of β-catenin in atopic dermatitis.

TNF-α binds to keratinocytes, and this binding activates hyperproliferation and adhesion molecules through NFκB-dependent pathways.9 Moreover, IL-1β released by epithelial cells is implicated in synthesis and release of cytokines and adhesion molecules as well as enhances development of pain, fever and hypotension.10 Crocin was previously reported to ameliorate inflammation in many pathological conditions as collagen-induced arthritis in rats, malathion-induced Parkinson-like behavior in rats and doxorubicin-induced myocardial toxicity in rats.11-13 However, this is the first study to discover its therapeutic effect in AD.
Figure 4: Effect of AD alone and in combination with 20 mg/kg crocin on (A) gene expression tumor necrosis factor (TNF)-α, (B) interleukin (IL)-1β, (C) IL-1β in skin of mice as well as (D) levels of TNF-α in skin of mice.

*: significant difference compared with the control groups at p<0.05. #: significant difference compared with atopic dermatitis group at p<0.05.

**CONCLUSION**

Authors can conclude that crocin could ameliorate DNCB-induced AD in mice. The current study explored for the first time the ability of crocin to block β-catenin with subsequent reduction in inflammatory pathway. The mechanism of the protective effects of crocin against atopic dermatitis was summarized in Figure 5.

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**Conflict of interest:** None declared

**Ethical approval:** The study was approved by the institutional ethics committee of University of Tabuk, Saudi Arabia

**REFERENCES**


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