

Original Research Article

Crocin ameliorated skin tissue inflammation in atopic dermatitis in mice

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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, NF κ B, TNF- α and IL-1 β .

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, NF κ B, TNF- α and IL-1 β .

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

Keywords: β -catenin, Interleukin -1 β , Nuclear factor κ B, 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.¹ Considered the superior cause of non-fatal condition in the skin, AD is estimated to influence about 230 million worldwide according to WHO.²

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.³ 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.⁴

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.⁵ 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/NF κ B 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.⁶ 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.

Primer	Accession number	Sequence (sense, antisense)
TNF-α	X02611	5'- TACTGAACCTTCGGGGTGATTGGTCC -3' 5'-CAGCCTTGTCCCTTGAAGAGAACC -3'
IL-1β	NM_008361	5'- AGTTGACGGACCCCAAAGAT -3' 5'- GGACAGCCCAGGTCAAAGG -3'
NFκB	NM_008689	5'-GAAATTCCTGATCCAGACAAAAAC-3' 5'-ATCACTTCAATGGCCTCTGTGTAG-3'
GAPDH	M32599	5'- ACCACAGTCCATGCCATCAC -3' 5'- CACCACCCTGTTGCTGTAGCC -3'

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).

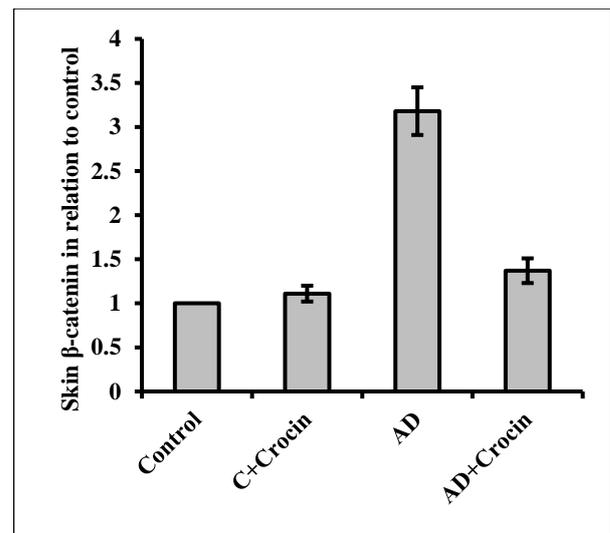
Statistical analysis

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 0.034±0.004 mm, respectively). However, crocin ameliorated number of scratches (5.97±0.52 scratches/10minutes), dermatitis score (2.9±0.26) and ear thickness (0.13±0.012 mm) without affecting control group (Figure 2).

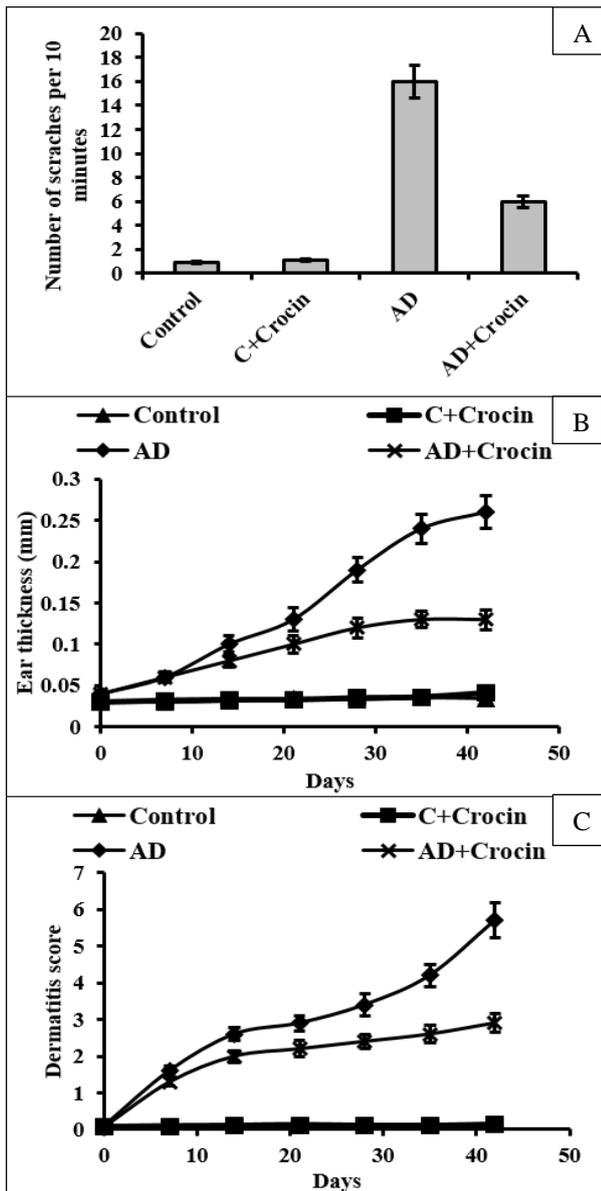


Figure 2: Effect of AD alone and in combination with 20 mg/kg crocin on skin levels of (A) number of scratched per 10 minutes (B) dermatitis score and (C) ear thickness.

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

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).

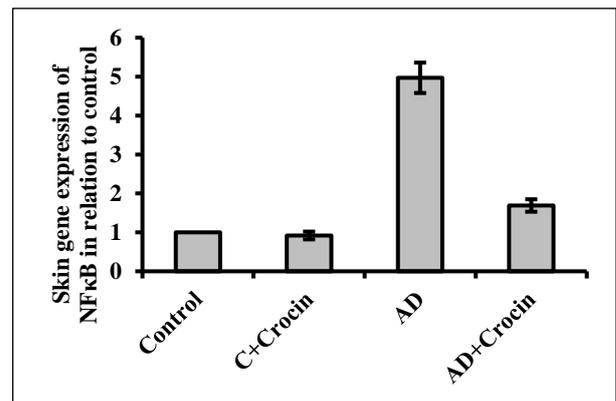


Figure 3: Effect of AD alone and in combination with 20 mg/kg crocin on gene expression of nuclear factor (NF)κB.

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

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.

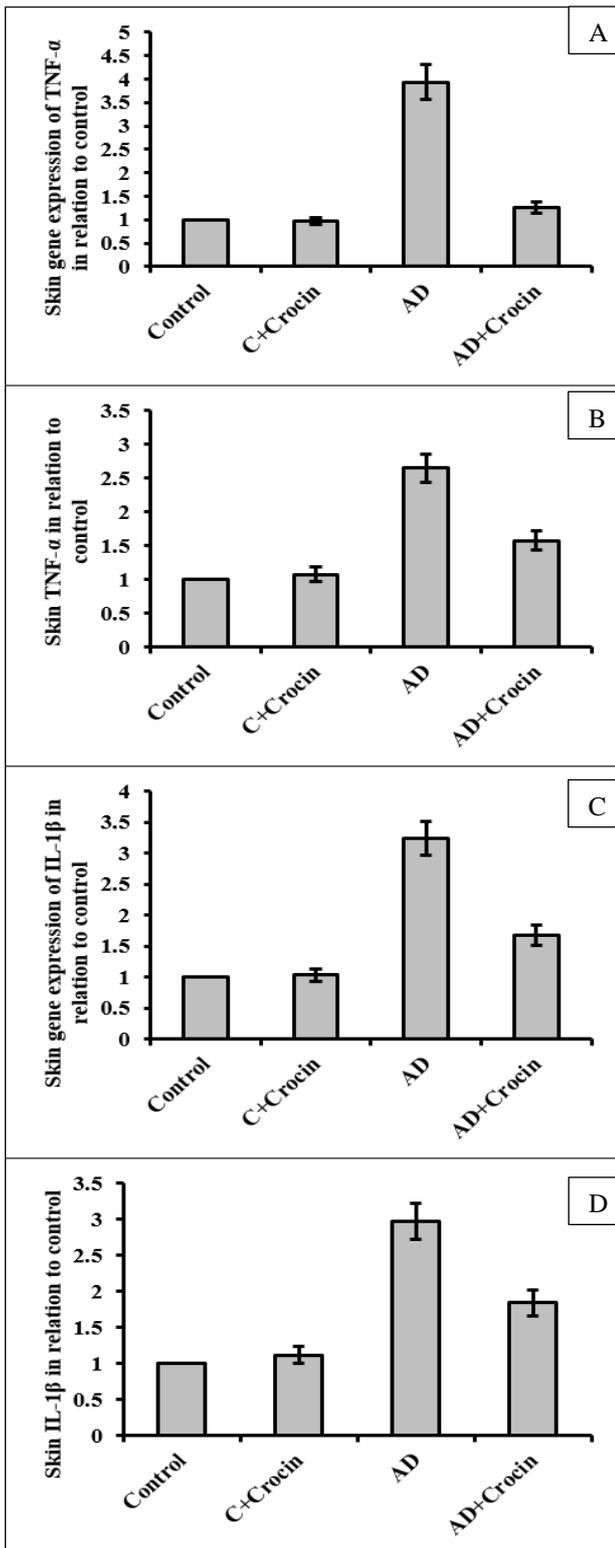


Figure 4: Effect of AD alone and in combination with 20 mg/kg crocin on (A) gene expression tumor necrosis factor (TNF)- α , (B) interleukin (IL)-1 β , (C) IL-1 β in skin of mice as well as (D) levels of TNF- α in skin of mice.

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

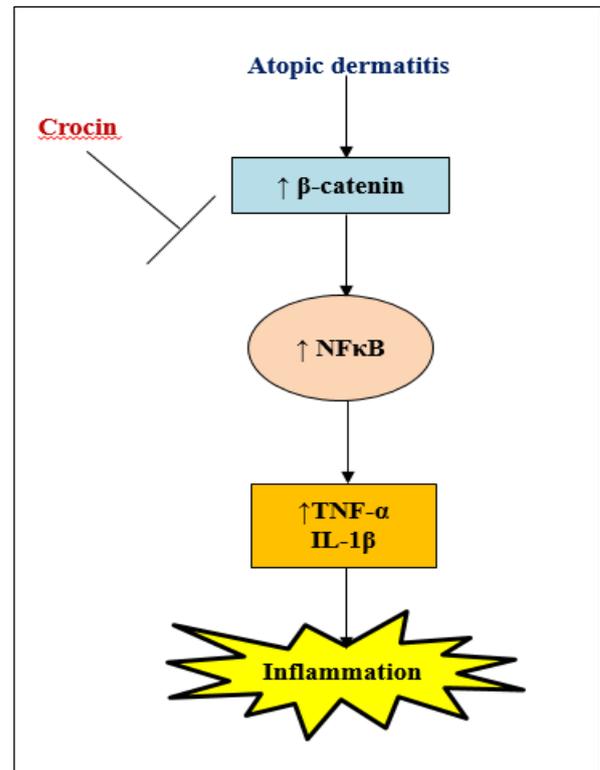


Figure 5: Mechanism of the protective effects of crocin against atopic dermatitis in mice.

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

1. Torres T, Ferreira EO, Goncalo M, Mendes-Bastos P, Selores M, Filipe P. Update on Atopic Dermatitis. *Acta medica portuguesa.* 2019;32:606-13.
2. Weidinger S, Beck LA, Bieber T, Kabashima K, Irvine AD. Atopic dermatitis. *Nature Rev Dis Prim.* 2018;4:1.
3. Silverberg JI, Gelfand JM, Margolis DJ, Boguniewicz M, Fonacier L, Grayson MH, et al. Health Utility Scores of Atopic Dermatitis in US Adults. *J Aller Clin Immunol Pract.* 2019;7:1246-52.
4. Boguniewicz M, Alexis AF, Beck LA, Block J, Eichenfield LF, Fonacier L, et al. Expert perspectives on management of moderate-to-severe atopic dermatitis: a multidisciplinary consensus

- addressing current and emerging therapies. *J Aller Clin Immunol: Pract.* 2017;5(6):1519-31.
5. Jung WS, Chae YS, Kim DY, Seo SW, Park HJ, Bae GS, et al. Gardenia jasminoides protects against cerulein-induced acute pancreatitis. *World J Gastroenterol: WJG.* 2008;14:6188-94.
 6. Alyoussef A. Arjunolic acid protects against DNCB-induced atopic dermatitis-like symptoms in mice by restoring a normal cytokine balance. *Eur Cytok Network.* 2015;26:38-45.
 7. Sung YY, Lee AY, Kim HK. The Gardenia jasminoides extract and its constituent, geniposide, elicit anti-allergic effects on atopic dermatitis by inhibiting histamine in vitro and in vivo. *J Ethnopharmacol.* 2014;156:33-40.
 8. Park JH, Lee KY, Park B, Yoon J. Suppression of Th2 chemokines by crocin via blocking of ERK-MAPK/NF-kappaB/STAT1 signalling pathways in TNF-alpha/IFN-gamma-stimulated human epidermal keratinocytes. *Exper Dermatol.* 2015;24:634-6.
 9. Moy AP, Murali M, Kroshinsky D, Horn TD, Nazarian RM. T-helper immune phenotype may underlie 'paradoxical' tumour necrosis factor-alpha inhibitor therapy-related psoriasiform dermatitis. *Clin Exper Dermatol.* 2018;43:19-26.
 10. Alshevskaya AA, Lopatnikova JA, Krugleeva OL, Nepomnyschih VM, Lukinov VL, Karaulov AV, et al. Expression density of receptors to IL-1beta in atopic dermatitis. *Mol Immunol.* 2016;75:92-100.
 11. Liu W, Sun Y, Cheng Z, Guo Y, Liu P, Wen Y. Crocin exerts anti-inflammatory and anti-arthritis effects on type II collagen-induced arthritis in rats. *Pharma Biol.* 2018;56:209-16.
 12. Mohammadzadeh L, Hosseinzadeh H, Abnous K, Razavi BM. Neuroprotective potential of crocin against malathion-induced motor deficit and neurochemical alterations in rats. *Envir Sci Pollut Res Inter.* 2018;25:4904-14.
 13. Elsherbiny NM, Salama MF, Said E, El-Sherbiny M, Al-Gayyar MM. Crocin protects against doxorubicin-induced myocardial toxicity in rats through down-regulation of inflammatory and apoptic pathways. *Chemico-biol Inter.* 2016;247:39-48.

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