

## The effects of crocin, mesalazine and their combination in the acetic acid-induced colitis in rats

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Article Info	Abstract
<b>Article history:</b>  Received: 09 April 2018 Accepted: 22 May 2018 Available online: 15 September 2019	<p>Crocin, as a carotenoid compound of saffron, exerts a potent antioxidant property. Mesalazine is frequently used in the treatment of ulcerative colitis. This study investigated the effects of separated and combination treatments with crocin and mesalazine in a rat model of ulcerative colitis. Ulcerative colitis was induced by intra-colonic administration of acetic acid (4.00%, 1.00 mL) at 8 cm proximal of the anus. Normal saline, acetic acid, crocin (5.00, 10.00 and 20.00 mg kg<sup>-1</sup>), mesalazine (100 and 300 mg kg<sup>-1</sup>) and crocin (5.00 mg kg<sup>-1</sup>) plus mesalazine (100 mg kg<sup>-1</sup>) were administered after induction of colitis for eight days. Body weight, organosomatic index (OSI), macroscopic and microscopic evaluations of colon and measurement of malondialdehyde (MDA), superoxide dismutase (SOD), tumor necrosis factor-alpha (TNF-α) contents of colon tissue were determined on day eight after induction of colitis. Crocin (10.00 and 20.00 mg kg<sup>-1</sup>), mesalazine (300 mg kg<sup>-1</sup>) and crocin (5.00 mg kg<sup>-1</sup>) plus mesalazine (100 mg kg<sup>-1</sup>) significantly (<math>p &lt; 0.05</math>) improved body weight and OSI and reduced macroscopic and microscopic scores. These treatments also significantly (<math>p &lt; 0.05</math>) recovered the increased levels of MDA and TNF-α as well as the decreased level of SOD in colon tissue. Crocin and mesalazine did not produce significant effects in intact rats. Based on the results, it is concluded that crocin and mesalazine produced protective effects on colon tissue via antioxidant and anti-inflammatory actions. In addition, a synergistic effect was observed between crocin and mesalazine in attenuating ulcerative colitis.</p>
<b>Key words:</b>  Crocin Mesalazine Rats Ulcerative colitis	

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### Introduction

Saffron, the most expensive herb spice, is obtained from the respective flower's stigmas of *Crocus sativus*.<sup>1</sup> In addition to its use as a spice, saffron has long been considered as an important medicinal plant for its therapeutic properties.<sup>2</sup> The most important bioactive components of saffron are safranal, crocetin and crocin.<sup>3</sup> Crocin is the unique water-soluble carotenoid found in saffron and the primary component contributing to the bright red color of saffron.<sup>4</sup> In addition to exerting a potent antioxidant activity, this carotenoid possesses tissue protective effects in various tissues such as nervous and gastrointestinal systems.<sup>5,6</sup>

Ulcerative colitis, a subtype of inflammatory bowel disease, is characterized by rectal bleeding, diarrhea,

tenesmus, and abdominal pain.<sup>7</sup> Although the etiology of ulcerative colitis is not well-understood, recent studies have suggested genetics, gastrointestinal immunity and environment involvements in the pathophysiology of ulcerative colitis.<sup>8,9</sup> Current treatment of ulcerative colitis is based on the use of four basic drug classes: amino-salicylates (5-ASA), steroids, immunosuppressants, and biologic therapies.<sup>10</sup> Due to various side effects of these drugs, a great number of studies in recent years have suggested the importance of medicinal plants and their bioactive substances in treating this condition.<sup>11,12</sup>

During the last decades, a large number of experimental models of chemically-ulcerative colitis have been developed. These models have proved to be helpful tools for obtaining new insights in the pathogenesis of ulcerative colitis and for the preclinical evaluation of new

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therapies.<sup>13</sup> Among these models, trinitrobenzene sulfonic acid (TNBS)-, oxazolone (OZ)-, dextran sulphate sodium (DSS)-, and acetic acid (AA)-induced colitis are most widely used to induce inflammation and ulceration in the rectum and the colon in rodents.<sup>14</sup>

Recently, it has been reported that crocin suppresses DDS-induced colitis by inhibiting inflammation.<sup>15</sup> On the other hand, mesalazine is most often used for ulcerative colitis therapy.<sup>16</sup> In addition, combination therapy with antioxidants and mesalazine produced better-improving effects.<sup>17,18</sup> Based on these evidences, the present study was aimed to investigate the possible tissue protective effects of crocin and mesalazine in separate and in combined treatments against acetic acid-induced ulcerative colitis by body weight, colon weight, colon macroscopic and microscopic and tissue biochemical evaluations.

## Materials and Methods

**Animals.** Healthy adult male Wistar rats (220-250 g) were used throughout the study. The animals were maintained in a laboratory under controlled 12 hr light-dark cycle and ambient temperature ( $22.00 \pm 0.50^\circ\text{C}$ ) with *ad libitum* food and water. All experiments were performed between 10:00 hr and 16:00 hr. The research and animal care procedures were approved by Veterinary Ethics Committee of the Faculty of Veterinary Medicine of Urmia University (AECVU-156-2018) and were performed in accordance with the National Research Council Guide for Care and Use of Laboratory animals.

**Chemicals.** Crocin powder was purchased from Sigma-Aldrich Co. (St Louis, USA). Mesalazine (Iran Hormone Co., Tehran, Iran), Ketamine 10.00% (Alfasan, Woerden, Holland) and xylazine 2.00% (Alfasan) solutions were purchased from a native drug store. Analytical chemical compounds such as thiobarbituric acid and acetic acid were purchased from Merck Co. (Dusseldorf, Germany).

**Experimental groups.** Forty-eight male Wistar rats were divided into eight groups of six rats each. Group 1, intact group, was administered with normal saline after intra-colonic administration of normal saline. Group 2, colitis group, received normal saline after intra-colonic administration of acetic acid. Groups 3, 4 and 5, crocin groups, were treated with 5.00, 10.00 and 20.00 mg kg<sup>-1</sup> crocin for eight days after induction of colitis, respectively. Groups 6 and 7, mesalazine groups, were treated with mesalazine (100 and 300 mg kg<sup>-1</sup>) for eight days after induction of colitis, respectively. Group 8, crocin plus mesalazine group, received a combined treatment with low doses of crocin (5.00 mg kg<sup>-1</sup>) and mesalazine (100 mg kg<sup>-1</sup>) for eight days after induction of colitis. Normal saline and crocin were intraperitoneally (IP) injected at the volume of 1.00 mL kg<sup>-1</sup>. Mesalazine suspension was prepared in normal saline and was administered by gavage at a constant volume of 0.30 mL

per rat. Crocin and mesalazine doses used here were designed according to previous studies in which crocin (5.00, 10.00 and 20.00 mg kg<sup>-1</sup> for four weeks) and mesalazine (100 and 300 mg kg<sup>-1</sup> for seven days) were used.<sup>19,20</sup> In addition to these groups, another 12 rats were divided into four groups for clarifying the effects of 10.00 and 20.00 mg kg<sup>-1</sup> crocin and 100 and 300 mg kg<sup>-1</sup> mesalazine in the intact rats. The results of these groups were compared to the intact group.

**Induction of colitis.** Colitis was induced by intra-colonic instillation of 4.00% acetic acid.<sup>21</sup> After a 24 hr fasting, each rat was anesthetized with IP injection of a mixture of 50.00 mg kg<sup>-1</sup> ketamine (Alfasan) and 5.00 mg kg<sup>-1</sup> xylazine (Alfasan). A polyethylene catheter, with an inner diameter of 1.00 mm, was lubricated with liquid paraffin and was then inserted into the lumen of the colon via the anus. The catheter was advanced so that the tip was 8.00 cm proximal to the anus. Initially, each rat received 1.00 mL of normal saline flush followed by manual palpation of the abdomen to remove any feces. Then, 1.00 mL of 4.00% acetic acid (v/v in normal saline) was slowly infused into the colon and the rat was then maintained in a head-down position for 30 sec to limit expulsion of the solution. Finally, each rat received 1.00 mL of colonic wash containing normal saline. Control rats were treated identically but instead of the acetic acid, they received 1.00 mL normal saline infusion.

**Body weight.** Body weight was measured before and on days 2, 4, 6 and 8 after intra-colonic administrations of normal saline and acetic acid. Colon weight was measured after removal for calculation of oraganosomatic index (OSI) according to the following formula:<sup>22</sup>

$$OSI_{(colon)} = 100 \times \text{colon weight (g)} / \text{body weight (g)}$$

**Tissue collection.** On day eight after induction of colitis, the rats were decapitated, the abdominal wall was opened, and the colon with proximal rectum was removed. Thereafter, the mucosal surface of all tissue specimens were washed using cool normal saline, dried, weighed and macroscopically scored. Immediately, each specimen was longitudinally divided into two halves, one half for histopathological and another for biochemical evaluations. For histopathological evaluation, colon segments were fixed in 10.00% buffer formal saline, and for biochemical assay, the specimens were rinsed in ice-cold saline solution.

**Macroscopic scoring.** Macroscopic scoring of colon was performed on a 0-5 scale procedure described previously,<sup>23</sup> as follows: 0 = no damage, 1= localized edema and hyperemia, however, no ulcers or erosions, 2= slight bleeding or erosion, 3= ulcers or erosions with moderate edema, 4= severe ulceration, erosion, edema and tissue necrosis, and 5= two or more major sites of inflammation and ulceration extending > 1 cm along the length of colon.

**Microscopic scoring.** The formalin-fixed colons were dehydrated and embedded in paraffin and sliced into 5  $\mu$ m sections. The sections were hydrated and stained with Hematoxylin and Eosin (H & E). The microscopic scoring was done according to the previous method,<sup>24</sup> for edema, inflammation and crypt damage severities. The edema severity scores were: 0 = edema absent in the colon; 1 = mild edema in the mucosa; 2 = edema in the mucosa and sub-mucosa; 3 = edema in the entire wall of the colon; and 4 = severe edema in the entire wall of the colon. The inflammation severity scores were: 0 = none; 1 = mild; 2 = moderate; and 3 = severe. The crypt damage scores were: 0 = none; 1 = basal 1/3 damaged; 2 = basal 2/3 damaged; 3 = crypts lost and surface epithelium present; and 4 = crypts and epithelium lost.

**Biochemical assay.** The biochemical assay was made on colon tissue homogenates. The colon tissue segments were cut into small pieces and homogenized at 4 °C in 2 mL of ice-cold saline with a glass homogenizer. The resulting homogenate was passed through a cellulose filter to remove impurities and divided into aliquots for biochemical analysis. Colon tissue MDA level was measured by the thiobarbitoric (TBA) acid method.<sup>25</sup> Peroxidation was measured as the production of MDA, which in combination with TBA forms a pink chromogen compound whose absorbance was measured spectrophotometrically (UV-975; Jasco, Tokyo, Japan) at 532 nm. Colon tissue MDA results were expressed as nmol per mg protein. The SOD activity of colon tissue was determined by superoxide dismutase assay kit (Cayman Chemical, Ann Arbor, USA) according to the manufacture's instruction and was expressed as U per mg protein. Colon tissue content of TNF- $\alpha$  was measured by ELISA assay according to the kit instruction (Rat TNF- $\alpha$ ; eBioscience Inc., San Diego, USA) and expressed as pg per mg protein.

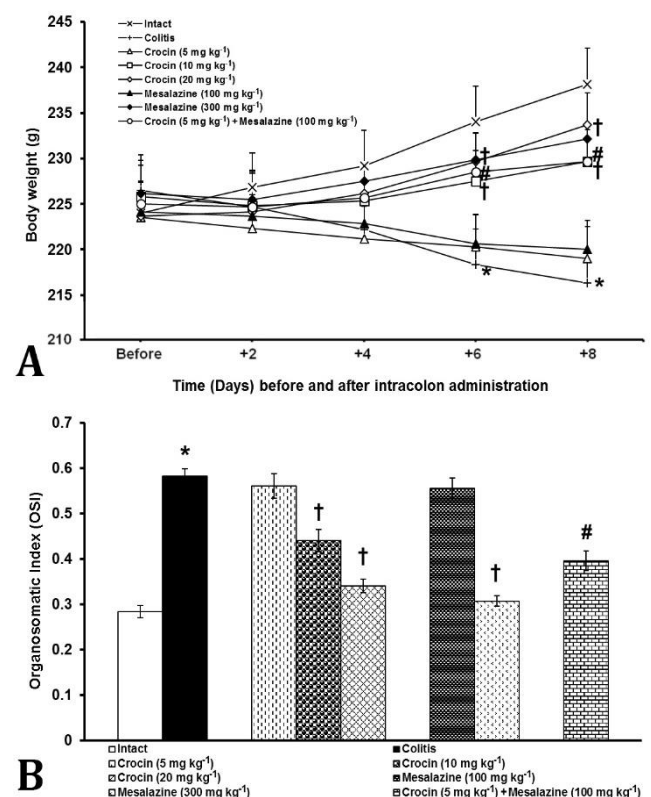
**Statistical analysis.** Statistical comparisons were performed using GraphPad Prism (version 5.0; GraphPad software Inc., San Diego, USA). Significance of body weight was assessed by two-way repeated measure analysis of variance (ANOVA) followed by Bonferroni post hoc test. Because of semi-quantitative nature of data obtained from macroscopic and microscopic alterations, Kruskal-Wallis and post hoc Mann-Whitney tests were applied. Colon weight and biochemical values were analyzed by one-way ANOVA, and post hoc Tukey's test. Significance at  $p < 0.05$  has been given receptive in all tests.

## Results

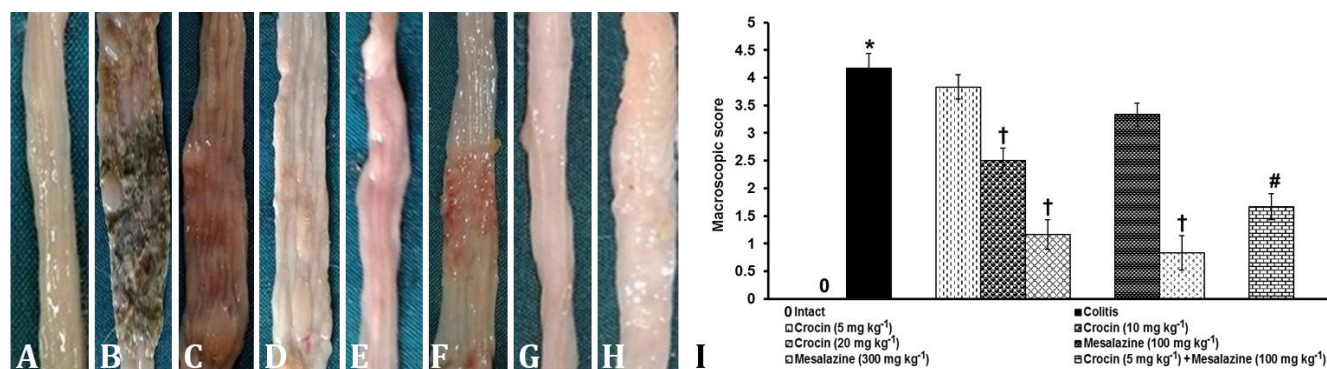
No significant differences were observed among intact groups treated with normal saline, crocin (10.00 and 20.00 mg kg<sup>-1</sup>) and mesalazine (100 and 200 mg kg<sup>-1</sup>), except for a non-significant elevation of SOD in crocin (20.00 mg kg<sup>-1</sup>) and a non-significant reduction of TNF- $\alpha$  in mesalazine (300 mg kg<sup>-1</sup>) treated groups.

In all groups, body weight showed no significant changes on the day before and on days 2 and 4 after intra-colonic administration of saline and acetic acid. Body weight of intact group was significantly ( $p < 0.05$ ) increased, whereas significant ( $p < 0.05$ ) reductions of body weight in colitis group were observed on days 6 and 8 after intra-colonic administration of acetic acid (Fig. 1A). Crocin (5.00 mg kg<sup>-1</sup>) and mesalazine (100 mg kg<sup>-1</sup>) did not alter, whereas, 10.00 and 20.00 mg kg<sup>-1</sup> crocin and 100 mg kg<sup>-1</sup> mesalazine significantly recovered the decreased body weight (Fig. 1A). A combined treatment with low doses of crocin and mesalazine produced a similar improving effect on the body weight (Fig. 1A). The OSI was significantly ( $p < 0.05$ ) increased in the colitis group. Crocin (10.00 and 20.00 mg kg<sup>-1</sup>) and mesalazine (100 mg kg<sup>-1</sup>) and their low dose combination treatments significantly ( $p < 0.05$ ) decreased the increased OSI induced by colitis (Fig. 1B).

Intact colon showed no damage (Fig. 2A) and macroscopic score 0 (Fig. 2I). The mucosal surface of the colon showed ulcer and erosion in the colitis group (Fig. 2B) and this group reached the macroscopic score to approximately 4.20 (Fig. 2I).



**Fig. 1.** Effects of separated and combination treatments with crocin and mesalazine on body weight (A) and organosomatic index (B) in acetic acid-induced colitis. Data are the mean  $\pm$  SEM from six rats in each group. \*  $p < 0.05$  compared to intact group, †  $p < 0.05$  compared to colitis group, #  $p < 0.05$  compared to colitis, crocin (5.00 mg kg<sup>-1</sup>) and mesalazine (100 mg kg<sup>-1</sup>) groups.

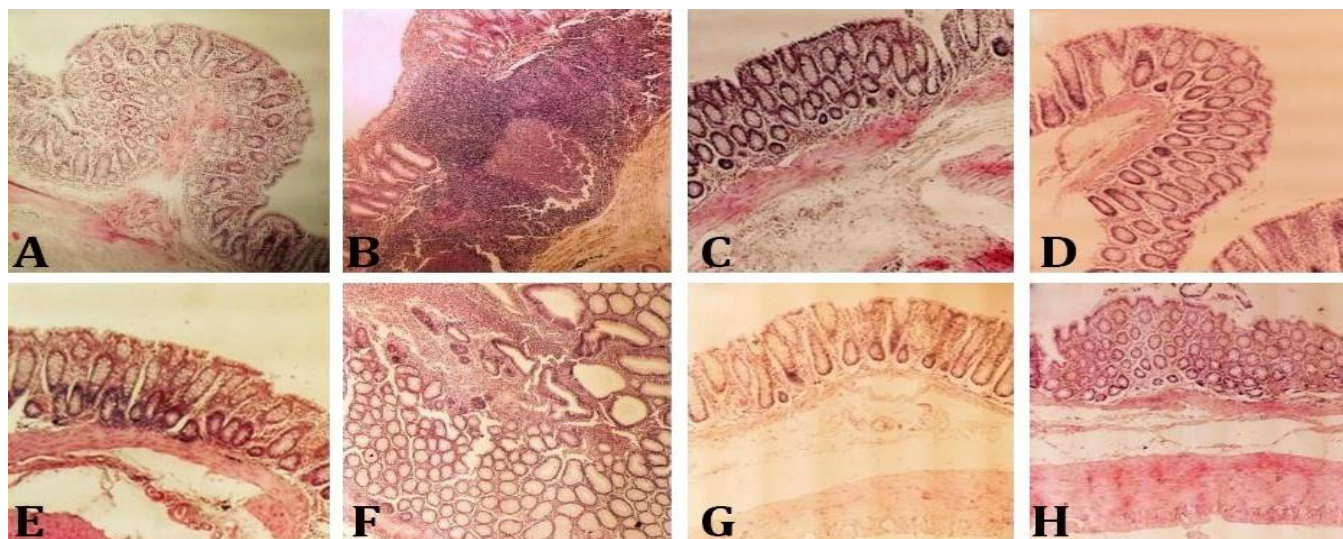


**Fig. 2.** Photos of colon mucosal surface in experimental groups. Intact: the mucosal surface was normal (A). Colitis: ulcers of various sizes are seen on the mucosal surface, the ulcers are so extensive that denudation of mucosal layer is evident and the bowel wall is thickened (B). Crocin 5.00 mg kg<sup>-1</sup>: hyperemia and small areas of erosions are seen (C). Crocin 10.00 mg kg<sup>-1</sup>: the mucosa is slightly hyperemic, with no ulcer or erosion (D). Crocin 20.00 mg kg<sup>-1</sup>: the mucosal surface appears normal (E). Mesalazine 100 mg kg<sup>-1</sup>: congested mucosal surface with foci of hemorrhage (F). Mesalazine 300 mg kg<sup>-1</sup>: slight hyperemia is seen (G). Crocin 5.00 mg kg<sup>-1</sup> plus mesalazine 100 mg kg<sup>-1</sup>: the mucosal surface is slightly hyperemic with no ulcer or erosion (H). (I) shows the macroscopic scores of colon mucosal surface in experimental groups. Data are the mean ± SEM from six rats in each group. \*  $p < 0.05$  compared to intact group, †  $p < 0.05$  compared to colitis group, #  $p < 0.05$  compared to colitis, crocin (5.00 mg kg<sup>-1</sup>) and mesalazine (100 mg kg<sup>-1</sup>) groups.

Crocin at a dose of 5.00 mg kg<sup>-1</sup> (Figs. 2C and 2I) and mesalazine (Figs. 2F and 2I) produced no significant effects on mucosal surface pathology and macroscopic score induced by colitis. Crocin at doses of 10.00 and 20.00 mg kg<sup>-1</sup> (Figs. 2D and 2E), mesalazine (300 mg kg<sup>-1</sup>; Fig. 2G) and crocin (5.00 mg kg<sup>-1</sup>) plus mesalazine (100 mg kg<sup>-1</sup>) (Fig. 2H) significantly ( $p < 0.05$ ) attenuated mucosal ulcer severity and decreased the increased microscopic score induced by colitis (Fig. 2I).

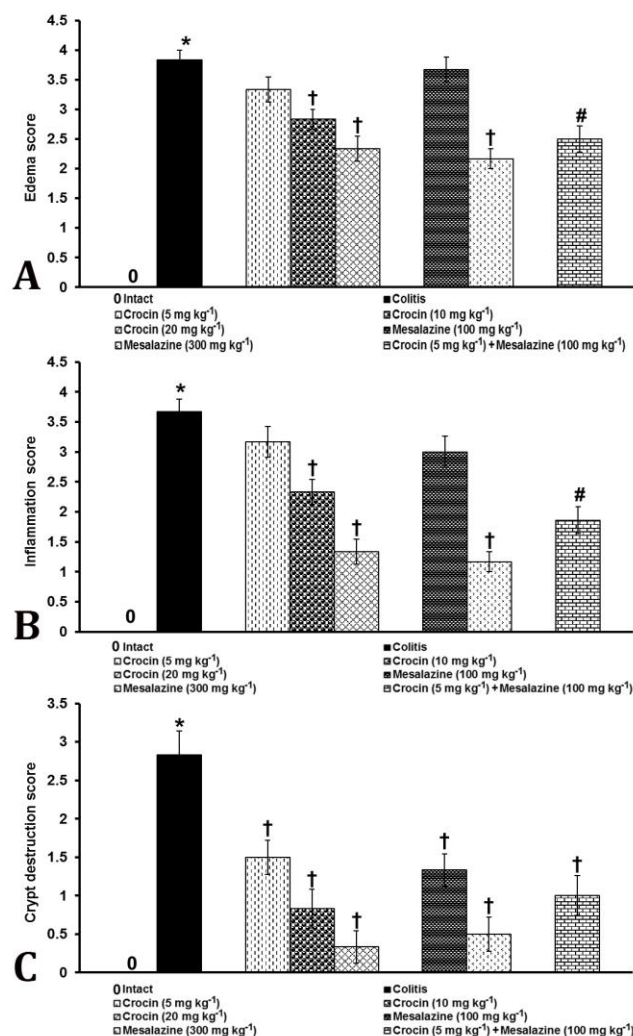
Intact colon showed normal architecture (Fig. 3A) and microscopic score 0 (Fig. 4). Colitis group showed

extensive edema, inflammatory cell infiltration and crypt destruction (Fig. 3B) and reached the respective scores to 3.80 (Fig. 4A), 3.50 (Fig. 4B) and 2.80 (Fig. 4C), respectively. These histopathological changes were not happened by 5.00 mg kg<sup>-1</sup> crocin (Figs. 3C, 4A, 4B, and 4C) as well as 100.00 mg kg<sup>-1</sup> mesalazine (Figs. 3F, 4A, 4B, and 4C). Crocin at doses of 10.00 and 20.00 mg kg<sup>-1</sup> (Figs. 3D, 3E, 4A, 4B and 4C), mesalazine (300 mg kg<sup>-1</sup>, Fig. 3G, 4A, 4B and 4C) and 5.00 mg kg<sup>-1</sup> crocin plus 100.00 mg kg<sup>-1</sup> mesalazine (Figs. 3H, 4A, 4B and 4C) significantly ( $p < 0.05$ ) attenuated colitis-induced histopathological changes.



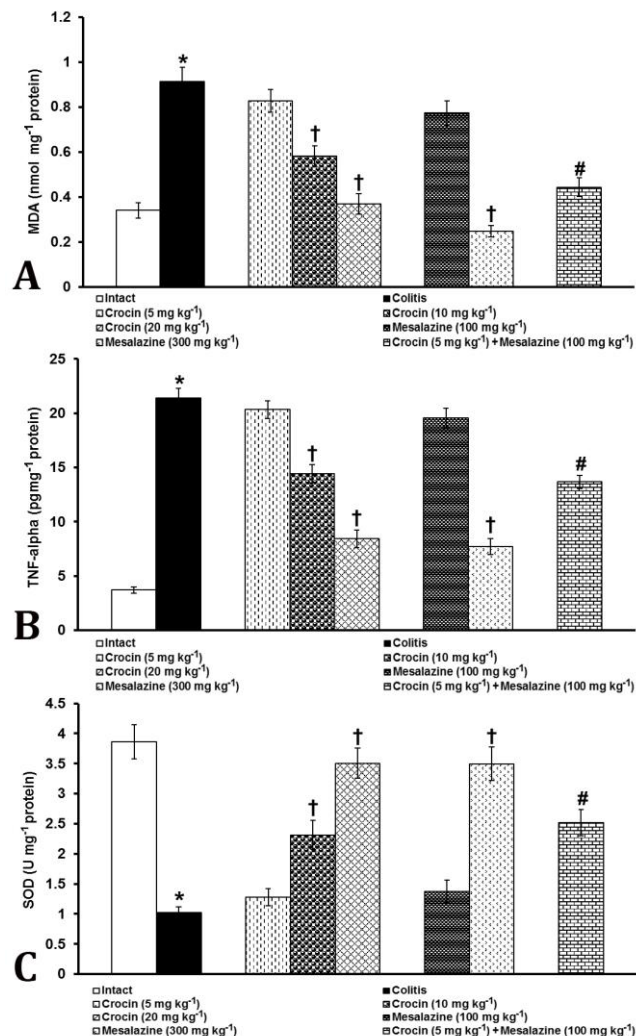
**Fig. 3.** Photomicrographs of colon tissue sections of experimental groups. (A) shows the intact normal architecture. Colitis: shows mucosal layer destruction, covered with a thick layer of fibrin. Submucosal edema and extensive inflammatory cell infiltration, and cryptoabscess were present with unequivocal crypt destruction. (B). Crocin 5.00 mg kg<sup>-1</sup>: leukocyte infiltration and degenerative crypts (C). Crocin 10.00 mg kg<sup>-1</sup>: shows moderate leukocyte infiltration (D). Crocin 20.00 mg kg<sup>-1</sup>: shows a slight leukocyte infiltration (E). Mesalazine 100 mg kg<sup>-1</sup>: shows submucosal edema, crypt destruction and extensive leukocyte infiltration (F). Mesalazine 300 mg kg<sup>-1</sup>: shows mild leukocyte infiltration (G). Crocin 5.00 mg kg<sup>-1</sup> plus mesalazine 300 mg kg<sup>-1</sup>: shows moderate leukocyte infiltration (H), (H&E, 100×).





**Fig. 4.** A, B, and C show scores of edema, inflammation and crypt destruction, respectively. Data are the mean  $\pm$  SEM from six rats in each group. \*  $p < 0.05$  compared to intact group, †  $p < 0.05$  compared to colitis group, #  $p < 0.05$  compared to colitis, crocin (5.00 mg kg<sup>-1</sup>) and mesalazine (100 mg kg<sup>-1</sup>) groups.

Colon tissue MDA and TNF- $\alpha$  levels and SOD activity in the intact group were  $0.34 \pm 0.03$  nmol mg<sup>-1</sup> protein,  $3.71 \pm 0.31$  pg per mg protein and  $3.86 \pm 0.38$  U per mg protein, respectively. In colitis group, MDA, TNF- $\alpha$  levels were significantly ( $p < 0.05$ ) increased to  $0.91 \pm 0.06$  nmol per mg protein,  $21.39 \pm 0.91$  pg per mg protein, respectively, and SOD activity was significantly ( $p < 0.05$ ) decreased to  $1.03 \pm 0.09$  U per mg protein. Crocin at a dose of 5.00 mg kg<sup>-1</sup> and mesalazine (100 mg kg<sup>-1</sup>) were without effect, whereas crocin at doses of 10.00 and 20.00 mg kg<sup>-1</sup>, mesalazine at a dose of 300 mg kg<sup>-1</sup> and low doses combination treatment with crocin and mesalazine significantly ( $p < 0.05$ ) reduced the increased levels of MDA and TNF- $\alpha$  significantly ( $p < 0.05$ ) elevated the decreased SOD activity in colon tissue induced by colitis (Fig. 5).



**Fig. 5.** Effects of treatments with crocin and mesalazine on MDA (A) and TNF- $\alpha$  (B) and SOD activity (C) in colon tissue in acetic acid-induced colitis. Data are the mean  $\pm$  SEM from six rats in each group. \*  $p < 0.05$  compared to intact group, †  $p < 0.05$  compared to colitis group, #  $p < 0.05$  compared to colitis, crocin (5.00 mg kg<sup>-1</sup>) and mesalazine (100 mg kg<sup>-1</sup>) groups.

## Discussion

The results of the present study showed that intra-colonic administration of acetic acid caused body weight loss, increase of colon weight, mucosal surface ulceration, colon wall edema, inflammatory cell infiltration in colon tissue, tissue level elevation of MDA and TNF- $\alpha$  and SOD activity reduction. Body weight loss confirmed in previous studies,<sup>23,24</sup> and may be related to diarrhea, anorexia, malabsorption, maldigestion, protein loss through the bowel<sup>26</sup> and alterations in the gastrointestinal absorptive functions.<sup>23,24</sup> The increase of colon weight has been reported by others,<sup>23,24</sup> and may be associated with mucosal hyperemia, extensive edema and expanded inflammation.<sup>27</sup> Macroscopic and microscopic changes of

colon wall observed in the current study were in consistence with other findings.<sup>28,29</sup> Elevation of MDA level, SOD activity reduction and TNF- $\alpha$  production expression have been reported after intra-colonic administration of acetic acid in rats.<sup>28-30</sup> Oxidative stress and inflammation have a long-lasting implication in both the etiology and the progression of ulcerative colitis.<sup>31,32</sup>

In the present study, crocin attenuated acetic acid-induced body and colon weight loss and macroscopic and microscopic alterations by reduction of MDA and TNF- $\alpha$  and elevation of SOD. In addition to various biological effects, saffron and its major constituents, crocin, crocetin, and safranal, have potent protective effects on the gastrointestinal system.<sup>6</sup> Treatment with crocetin attenuated TNBS-induced undesirable alterations such as neutrophil infiltration, MDA level and nitric oxide (NO) synthase expression in mice.<sup>33</sup> Only in one study, four weeks dietary feeding with crocin inhibited DDS-induced colitis and decreased mRNA expression of TNF- $\alpha$ , IL- $\beta$  and cyclooxygenase 2 in colon mucosa of male ICR mice.<sup>15</sup> In a rat model of ethanol-induced gastric ulcer, crocin improved gastric mucosal histopathological alterations, recovered mucosal levels of MDA, SOD, and TNF- $\alpha$ , and enhance gastric mucosal prostaglandin E2 (PGE2) production.<sup>34</sup> The results of the present study and other findings on digestive tract indicated that crocin could produce beneficial effects on gastrointestinal mucosa by its antioxidant and anti-inflammatory properties.

Our present results showed that treatment with mesalazine improved all body, organ, mucosal and biochemical alterations induced by intra-colonic administration of acetic acid. Mesalazine, also known as mesalamine, is a 5-aminosalicylic acid (5-aminosalicylate) compound and the most commonly used agent for ulcerative colitis therapy.<sup>16</sup> The exact mechanism of action of mesalazine remains poorly elucidated. It is believed that mesalazine exerts a negative effect on the cyclooxygenase and lipoxygenase pathways, thereby reducing the formation of pro-inflammatory prostaglandins and leukotrienes.<sup>35</sup> Mesalazine revealed anti-oxidative (by normalizing of oxidative stress markers) and anti-inflammatory (by decreasing of TNF- $\alpha$  level) effects in AA-induced colitis in rats.<sup>36</sup> Moreover, mesalazine improved body and colon weights, recovered histological alterations and normalized colon tissue levels of SOD, MDA, IL-1 $\beta$  and TNF- $\alpha$  in the rat model of DDS-induced colitis.<sup>37</sup> Therefore, the results of our study confirmed previous findings that showed colon tissue protective effects of mesalazine.

In this study, low doses of crocin and mesalazine produced a protective effect on colon tissue when used together, and that was comparable with the protective effects induced by high doses of crocin and mesalazine. There are no reports showing the combined effects of crocin and mesalazine in producing colitis outcomes. The combination of an antioxidant or anti-inflammatory agent

with an anti-colitis drug has proven to be more effective than monotherapy in their separate uses. For example, the inflammation induced by intra-colonic administration of TNBS was mitigated significantly by allicin (with anti-inflammatory and anti-oxidative properties) treatment, particularly combined with mesalazine.<sup>17</sup> In addition, n-acetylcysteine, an antioxidant agent, plus mesalazine exerted therapeutic benefit through counteracting PGE2 and the deleterious effects of oxidative and nitrosative stress induced by TNBS colitis.<sup>18</sup> Addition of curcumin, a potent antioxidant, and anti-inflammatory agent, to mesalamine therapy was superior to the combination of placebo and mesalamine in inducing clinical and endoscopic remission in patients with mild-to-moderate active ulcerative colitis, producing no apparent adverse effects.<sup>38</sup>

In conclusion, the results of the present study showed that intra-colonic instillation of acetic acid produced undesirable effects on body, colon and tissue biochemistry, in part by oxidative stress activation and inflammatory cytokine production. Separate and combination treatments with crocin and mesalazine produced colon tissue protective effects by recovering oxidative stress marker as well as TNF- $\alpha$  level. Combination treatment with crocin and mesalazine produced documented improving effect than those of separate use.

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## Conflicts of interest

The authors declare no conflict of interest.

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