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The Protective Effects of Honey and *Spirulina Platensis* on Acetic Acid-Induced Ulcerative Colitis in Rats

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Abstract

Background: Overproduction of reactive oxygen species (ROS) causes increased lipid peroxidation, decreased intestinal epithelial barrier integrity, and ultimately mucosal disruption and ulceration. Several studies have confirmed the antioxidant, anti-inflammatory, and immunomodulatory properties of *Spirulina Platensis* (SP), edible blue - green algae, in various inflammatory diseases. In addition, natural honey, a source of phenolic and flavonoid compounds, is a powerful antioxidant, which can help prevent chronic oxidative stress and subsequent inflammation.

Objectives: In this study, we examined and compared the protective effects of SP and honey on ulcerative colitis induced by acetic acid (AA) in rats.

Methods: Forty male rats were allocated into five groups (N = 8) and received pretreatment for 32 consecutive days. The administrations were as follows: group 1 (control) and group 2 (AA-colitis group): normal saline, group 3: 1 ml honey/day, group 4: 1 ml honey/day plus 1 g/kg SP, and group 5: 1 g/kg SP. Colitis was induced on the 30th day in groups 2 to 5. On day 32, the clinical activity score was determined and anesthetized animals were sacrificed. Serum interleukin-6 (IL-6), IL-1β, tumor necrosis factor-α (TNF-α), glutathione peroxidase (GPx), reduced glutathione (GSH), total antioxidant capacity (TAC), superoxide dismutase (SOD), myeloperoxidase (MPO), prostaglandin E2 (PGE2), malondialdehyde (MDA), nitric oxide (NO), and colonic weight/length ratio were determined. In addition, histopathological changes of the colon were observed microscopically.

Results: The inflammatory markers (PGE2, MDA, NO, IL-6, IL-1β, MPO, and TNF-α) were significantly lower in the pretreatment groups than in the AA-colitis group (P values < 0.05). PGE2 [median (IQR)] of the honey, SP + honey, and SP groups was [0.76 (0.33)], [0.75 (0.40)], and [0.87 (0.86)], respectively, compared to the AA-colitis group [2.60 (2.23)] (P values < 0.041). MDA values were [6.52 (3.57)], [6.09 (3.59)], and [5.85 (4.92)] vs. [16.60 (12.03)] (P values < 0.046) and IL-1β values were [42.20 (8.2)], [41.76 (18.10)], and [42.93 (14.09)] vs. [79.54 (40.79)] (P values < 0.044). Also, SOD, GSH, GPx, and TAC [median (IQR)] were significantly higher in the pretreatment groups than in the AA- colitis group (P values < 0.05). For example, TAC values of the honey, SP + honey, and SP groups were [0.164 (0.08)], [0.14 (0.05)], and [0.16 (0.10)], respectively, vs. the AA-colitis group [0.08 (0.01)] (P values < 0.028).

Conclusions: Honey and SP are favorable foods in preventing oxidative stress and inflammatory diseases such as ulcerative colitis.

Keywords: Antioxidant, Blue - Green Algae, Honey, Inflammation, Oxidative Stress, Spirulina, Ulcerative Colitis

1. Background

Inflammatory bowel disease (IBD) is an immune - mediated relapsing, chronic, gastrointestinal disease. The most common IBDs include Crohn's disease (CD) and ulcerative colitis (UC). UC involves the rectum and colon (1). In the past, IBD was known as a "Western" disease, while today, its prevalence is rising in Asian countries. The increasing prevalence is attributed to changes in lifestyle, especially dietary habits (2, 3). UC is related to an increase in colorectal malignancy that influences patients' quality of life (2).

The etiology and pathophysiology of IBD are not clearly understood. There is a consensus that IBD is a multifactorial disease. Interactions between environmental factors and genetic and immune responses may account for the overproduction of free radicals, reactive oxygen species (ROS), and proinflammatory cytokines, which start inflammatory responses to involve in the pathogenesis of IBD. An imbalance between oxidant/antioxidant systems results in oxidative stress and inflammatory cascade (4-6).

Inflammatory cell infiltration (mainly macrophages and neutrophils) during acute inflammation increases the formation of ROS and proinflammatory cytokines, such as TNF-α, IL-6, IL-1β, and inducible nitric oxide synthase

(iNOS) (7, 8). Overproduction of ROS, NO, and PGE2 leads to increased lipid peroxidation, decreased intestinal epithelial barrier integrity, and ultimately the mucosal disruption and ulceration (9, 10). Many immunogenic bacterial antigens from the damaged epithelial barrier enter submucosal layers and start a ruinous cascade of immune responses (11).

Environmental factors, such as dietary components, change the immune responses of the gut and intestinal microbiota. Some specific bacteria control the mucosal immune system and intestinal permeability. Bifidobacteria prevent microvillus damage, while pathogenic bacteria increase intestinal permeability (11, 12).

Dietary factors are highly effective modulators of intestinal microflora. Some dietary components increase the Gram-negative to Gram-positive bacteria ratio; therefore, a change in gut microbiota causes intestinal dysbiosis. Evidently, dysbiosis can be responsible for the elevated plasma bacterial lipopolysaccharide (LPS), which causes metabolic endotoxemia and consequently inflammation (2, 12).

High dietary intake of antioxidants has an inverse correlation with the risk of pathogenic conditions and inflammatory disorders due to oxidative stress. Dietary natural bioactive components with anti - inflammatory and antioxidant characteristics can suppress ROS - induced inflammatory responses, as well as excessive immune responses, and maintain homeostasis of the gastrointestinal tract and intestinal barrier function. Moreover, they can inhibit the NF-κB pathways and decrease proinflammatory cytokines. The consumption of potential chemopreventive dietary natural products, such as polyphenols, flavonoids, phycocyanin (PC), and γ-linolenic acid, in early life, could delay the UC onset and progression (2, 10, 13-16), therefore receiving a great deal of attention.

Previous studies have confirmed the antioxidant, anti-inflammatory, and immunomodulatory potential of SP in various inflammatory diseases (1, 17). Because of high nutritional values, SP has been traditionally used as food. It is rich in proteins (phycocyanin), minerals, vitamins, essential fatty acids, γ-linolenic acid, and phenolic acids (1, 16).

On the other hand, according to numerous studies, honey can be effective against wounds and UC (18, 19). In addition, natural honey, as a good source of phenolic and flavonoid compounds, is a powerful antioxidant, which may be effective in preventing chronic oxidative stress, as well as subsequent inflammation (5).

Most investigations have focused on the suppression of inflammation to relieve disease symptoms, delay relapses, or improve the secondary prevention of IBD. However, in the past few decades, new trends have been pursued to prevent oxidative stress and chronic inflammatory processes using natural dietary components, scilicet, and primary prevention (2). Consequently, identifying food sources and natural products, rich in antioxidant and inflammatory substances without any adverse effects, is critically needed to inhibit oxidative stress and consequent chronic inflammation. In this study, the protective effects of SP and honey against UC, induced by acetic acid, were examined in rats. This is the first long - term study of the concurrent oral administration of honey and SP against the animal model of UC by evaluating several inflammatory markers, antioxidants status, and histological changes.

2. Materials and Methods

2.1. Materials

Pure Hawaiian SP was prepared from Cyanotech Corporation (Kailua - Kona, Hawaii, USA). Honey was purchased from a store in Khorramabad (Lorestan Province, Iran). Rat PGE2 and MPO ELISA kits and GPX, GSH, MDA, SOD, TAC, and NO assay kits were provided by ZELLBio GmbH (Germany). In addition, ELISA kits for IL-6, IL-1β, and TNF-α were supplied from Diacolone SAS (France). Other chemicals were of analytical grade. In this study, before the use, all pieces of equipment were calibrated. They included Elisa Microplate Reader (Stat Fax 2100, AWARENESS, America), Elisa Microplate Washer (Stat Fax 2600, AWARENESS, America), Laboratory refrigerator Centrifuge (Sigma, 3K30, Germany), Analytical balances (AND, resolution 0.0001 gr, Japan), Incubator (Memmert, Germany), Freezer (New Brunswick Scientific, England), Medical homogenizer (Heidolph, Germany), and Microscope (Olympus Corporation BX41, model UDO3, Tokyo Japan).

2.2. Experimental Design

Male Sprague Dawley rats (N = 40) were purchased from the Center of Comparative and Experimental Medicine, Shiraz University of Medical Sciences, Shiraz, Iran. The rats were given one week before the experiment to become accustomed to the environment (humidity, 55 $\pm$ 10%; temperature, 22 $\pm$ 2 °C) in a 12:12 h light/dark cycle with free access to water and food. The animals (mean weight, 200 - 240 g) were allocated into five groups (N = 8) and placed in polypropylene cages under constant environmental conditions animals per cage). All groups received their respective pretreatments for 32 consecutive days via oral gavage. The groups were as follows:

Group 1: Normal control (NC) rats: receiving normal saline (2 ml) for 32 consecutive days and 2 ml normal saline intrarectally on the 30th day.

Group 2: Acetic acid - induced colitis or AA group: administered with normal saline (2 ml) for 32 consecutive

days and 2 ml acetic acid (3%, v/v) intrarectally on the 30th day.

Group 3: Honey or AA+H group: receiving 1 ml honey (diluted in 1 ml of distilled water) for 32 consecutive days and 2 ml of acetic acid (3%, v/v) intrarectally on the 30th day.

Group 4: Honey + Spurinula or AA + HS group: receiving 1 ml honey (diluted in 1 ml of distilled water) in the morning and 1 g/kg Spirulina (after a few hours; 2 ml suspension with distilled water) for 32 consecutive days, then receiving 2 ml acetic acid (3%, v/v) intrarectally on the 30th day.

Group 5: (Spuriluna or AA + S group): receiving 1 g/kg Spirulina (2 ml suspension with distilled water) for 32 consecutive days and 2 ml acetic acid (3%, v/v) intrarectally on the 30th day.

The scores of clinical activity of UC were determined by summing up the scores of stool consistency, bleeding, and weight loss within 48 hours after colitis induction, divided by 3 according to Thippeswamy et al. criteria (Table 1) (20). Ethyl ether was used to anesthetize the animals, and blood samples were obtained to perform biochemical analyses. Through cervical dislocation, the rats were sacrificed. The abdomen was opened, and the colon was dissected and split longitudinally. In the next step, it was cleaned with cold saline in order to remove fecal residues. Adherent adipose tissues were removed, and it was dried with a filter paper. The colon length and weight were measured and imaged. After separating the blood serum, it was stored at -75 °C until the assays. All possible efforts were made to reduce the animals' pain during the experiments. This research was approved by the ethics committee of Shiraz University of Medical Sciences (No. IR. SUMS.REC. 1394. S605), Shiraz, Iran.

2.3. Induction of Colitis

On the 28th day, the animals were deprived of food for 36 hours and received their oral gavages while they could freely access to tap water. On the 30th day, 1 hour after oral gavage, the animals were anesthetized via ethyl ether inhalation. Then, a polyethylene tube (diameter, 2 mm) was used to infuse 2 ml of AA (3%, v/v) into the colon. Through the rectum, the tube was inserted in the colon at an 8 - cm distance. To inhibit early intracolonic instillation leakage, the animals were maintained in the Trendelenburg position for 30 seconds.

2.4. Histopathological Study

For the histopathological examination, 10% formalin was used to fix 2 cm of the colon specimen. The samples were embedded in paraffin wax blocks. After cutting 5 - μm sections from the paraffin blocks, hematoxylin and eosin staining were applied. Histopathological changes were observed and photographed by a pathologist blindly according to the Dieleman et al. criteria (21). The remaining parts of the colon specimens were stored at a temperature of -75 °C for biochemical examinations.

Table 1. Clinical Activity Score

	Clinical Activity Score
Weight loss	No weight loss = 0
	1 to 5% = 1
	5 to 10% = 2
	10 to 20% = 3
	20% = 4
Stool consistency	Well - formed pellets = 0
	Pasty and semi-formed stools that did not stick to the anus = 2
	Liquid stools that stuck to the anus = 4
Bleeding of the colon	No blood in hemoccult = 0
	Positive hemoccult = 2
	Gross bleeding = 4
Total clinical score (TCS)	The combined score of weight loss, stool consistency, and bleeding divided by 3

2.5. Biochemical Analysis

Colonic tissues were homogenized, and MPO, MDA, NO, and PGE2 concentrations were measured in homogenized supernatants via ELISA assay as instructed by the manufacturer. GPX and SOD activities, as well as GSH and TAC, TNF-α, IL-6, and IL-1β levels in serum samples were determined with ELISA kits, as instructed by the manufacturer.

2.6. Statistical Analysis

Data are presented as median (Interquartile Range = IQR). SPSS version 16.0 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism version 6.07 (Graphpad Inc., San Diego, CA, USA) were used for analyzing the data. Normality of data was examined using Kolmogorov - Smirnov and Shapiro - Wilk tests. The distribution of data was not normal. Thus, for statistical analysis, nonparametric Kruskal - Wallis test, as well as Dunn's test, was applied. The significance level was set at 0.05.

3. Results

3.1. Colonic Weight / Length Ratio, Total Clinical Scores and Weight Loss after Colitis

Clinical signs of colitis (body weight loss, rectal bleeding, and diarrhea) were investigated to examine its severity. A significant increase in TCS was reported in colitis rats

in the AA - colitis and honey groups in comparison with the NC group ($P < 0.001$ and $P < 0.01$, respectively). Pretreatment with SP and honey significantly reduced TCS in the HS group compared to the AA - colitis group ($P < 0.041$). The SP and HS groups experienced no significant change in TCS compared to the NC group. Additionally, no significant change was seen in the colon length of the administered groups compared to the NC group. Nonetheless, the NC and honey groups showed significantly different weight/length ratio, compared to the AA - colitis group ($P < 0.001$ and $P < 0.013$, respectively) (Table 2).

3.2. Biochemical Studies

3.2.1. IL-6, TNF-α, and IL-1β

The serum levels of proinflammatory cytokines remarkably increased in all colitis groups in comparison with the NC group (P values < 0.05). Pretreatment with honey, SP, and honey + SP significantly reduced IL-1β, IL-6, and TNF-α level in comparison with the AA group (Figures 1A, 1B, and 1C). On the other hand, the IL-6 level was three times higher in the AA-colitis group than in the NC group ($P < 0.001$).

3.2.2. MPO, NO, MDA, and PGE2 Levels in Colon Tissues

The MPO level significantly increased in the colonic tissues of the AA-colitis group as compared with the other groups (Figure 1D). The NC and pretreatment colitis groups were not significantly different regarding the MPO level in colonic tissues. Preventive treatment with honey, SP, and the combination regimen significantly attenuated colonic tissues NO level, compared to the AA-colitis group (P values < 0.036), as depicted in Figure 1E.

In the AA-colitis group, the NO level of colonic tissues increased significantly (2.9 times) in comparison with the NC group (P value < 0.005). The MDA levels of colonic tissues increased (3.9 times) in the AA-colitis group versus the control group (P value < 0.001) (Figure 1F). Moreover, the AA-colitis group had a significantly increased PGE2 level in colonic tissues (5.8 times) in comparison with the NC group (P value < 0.001). A significant decline was reported in the PGE2 level of colonic tissues in all pretreatment groups in comparison with the AA group, as shown in Figure 1G (P values < 0.041).

3.2.3. Serum Antioxidant Status

In comparison with the NC and pretreatment colitis groups, AA-induced colitis caused a significant decline in serum enzymatic (SOD and GPx) and nonenzymatic (GSH) antioxidants in the AA group as shown in Figures 1H, 1I, and 1J (P values < 0.044). All pretreatment regimes showed effectiveness, although the NC and pretreatment colitis groups were not significantly different. The AA-colitis group showed a dramatic decline in the serum TAC level, compared to the HS, SP, and NC groups (P values < 0.03). In terms of TAC, no significant difference was found between the normal controls and H, HS, and SP groups (Figure 1K).

3.3. Histopathological Studies

The histological examination of colonic tissues in the NC group did not show any changes and a normal mucosal architecture was observed. In contrast, the AA-induced colitis group experienced severe acute inflammation extending to the muscular layer, hemorrhage, necrosis, ulceration, goblet cell depletion, and crypt architectural distortion. The histological assessment revealed that pre-administration of honey alone and co-administration of SP and honey in the honey and HS groups significantly decreased inflammation, cellular infiltration, mucosal ulceration, and edema, compared to the AA-colitis group. In the SP group, pretreatment with SP attenuated the severity and the extent of colitis and showed better protective effects. Rats in the SP group revealed mild mucosal inflammation, mild architectural gland disarray, and crypt hyperplasia (Figure 2).

4. Discussion

The findings of our study demonstrated pretreatment with honey and SP has protective effects against AA-induced UC among rats. The administration of honey and SP led to improvements in colon weight/length ratio, TCS, and serum antioxidants, namely SOD, GSH, GPX, and TAC. On the other hand, it caused a significant decline in NO, MPO, PGE2, and MDA levels in colon tissues and significantly reduced the serum levels of TNF-α, IL-1β, and IL-6 in all pretreatment groups, compared to the AA-colitis group. The protective activities of honey and SP were confirmed by histopathological evaluations; the antioxidant and anti-inflammatory characteristics could explain their protective effects. According to our knowledge, this is the first study reporting: 1) The long-term duration of pre-administration of SP, honey, and their combination in the prevention of colitis and their effects on a large number of pro-inflammatory cytokines, oxidative stress end-products, antioxidant enzymes and total antioxidant capacity, histological changes, and clinical activity scores of disease simultaneously. In previous studies, the duration of the intervention was 15 days for SP and four days for honey; 2) This is the first study examining the impact of oral honey pre-administration on colonic MPO, PGE2, NO, serum TAC, IL-6, IL-1β, and TNF-α levels, as well as SOD and GPX activities on experimental colitis; 3) It is

Table 2. The Effect of Pre-Administration of Honey, *Spirulina Platensis*, and Honey + Spirulina on Colon Weight/Length Ratio, Total Clinical Scores, and Weight Loss 48h after Colitis[a]

Parameter	Normal Control, (N = 8)	Acetic Acid, Induced Colitis, (N = 8)	Honey (N = 8)	Honey + Spirulina, (N = 8)	*Spirulina Platensis*, (N = 8)
Colon weight/length ratio	76.23 (13.30) [b]	161.62 (17.93)	96.65 (37.37) [b]	112.74 (41.33)	106.28 (20.28)
Total clinical scores (TCS)	.33 [b]	3.66 (1.08)	2.33 (.25) [c]	2.00 (.66) [b]	1.83 (1.16)
Weight loss 48h after colitis (g)	-9.50 (1.75) [b]	-46.00 (26.25)	-37.50 (15.25) [c]	-37.50 (31.50) [c]	-30.00 (30.50)

[a] Values expressed are median (IQR).
[b] Values significantly different from acetic acid - induced colitis group ($P < 0.05$).
[c] Values significantly different from the normal control group ($P < 0.05$).

also the first study evaluating the effects of oral pretreatment with SP on colonic NO and serum GPX and TAC levels on the animal model of colitis. Pathophysiological mechanisms and histopathological features of experimental AA-induced colitis are similar to human colitis. It increases the production of ROS and the consequent imbalance of oxidant/antioxidant substances causes inflammatory - oxidative - apoptotic cascade (22).

Spirulina is gaining attention due to its nutraceutical and potential pharmaceutical properties. It is an excellent source of a unique blend of potent antioxidant and anti-inflammatory nutrients such as phycocyanin, a protein that includes tetrapyrrole phycocyanobilin which is a strong antioxidant, gamma - linolenic acid, alfa - lipoic acid, ergothioneine, β-carotene, zeaxanthin, vitamin E, vitamin C, selenium, and zinc (23, 24).

SP exhibits its anti-inflammatory properties by increasing histone H3 acetylation and NF-kB inhibition, which reduce proinflammatory cytokines expression and secretion in the macrophages (25). Honey contains phenolic compounds, certain enzymes, amino acids, vitamins, carotenoids, and minerals (26). Antioxidant, anti-inflammatory, immune - modulatory, and analgesic effects and major antibacterial activities of honey are mainly attributed to its polyphenols. However, various active components in honey like flavonoids, phenolic acids, tocopherols, ascorbic acid, GSH, catalase, and SOD have synergetic effects on the antioxidant induction and inflammatory properties of natural honey. These unique properties identified in honey resulted in the use of honey as a chemopreventive agent for treatment and prevention of inflammatory disorders (18, 26).

In UC, the overproduction of IL-1β, IL-8, IL-6, and PGE2 causes epithelial cell necrosis and edema (27). Colonic tissue edema resulted in bowel wall thickening, reduced colon length, and increased colon wet weight and weight/length ratio (1, 20). In the present study, the administration of honey and SP individually and SP + honey decreased colonic shortening and colon weight/length ratio. However, this reduction only was significant in the honey group compared to the AA-colitis group. Honey and its extracts significantly decreased edema perhaps through the suppression of NO and PGE2 production as molecular vasodilators (28). Previous studies have not reported the effects of preadministration of honey alone and honey + SP on colon weight/length ratio. However, Abdel-Daim et al. reported a significant colon weight/length ratio reduction in rats receiving SP in comparison with an AA group (1). The duration and SP dosage were lower in their study than in ours.

We observed improved TCS in all pretreatment groups, but only TCS of the honey + SP group was significantly lower than that of the AA-colitis group. Spirulina showed better results in the inhibition of weight loss compared to honey alone and the combined regimen. However, honey offered better results in stool consistency. We did not find any similar study in the literature reporting the effects of preadministration of honey on TCS, weight loss, and colonic weight and length. However, a study administered honey for six weeks after colitis and reported weight loss and improved disease activity index (18). These findings in the case of the effects of spirulina on colonic weight/length ratio are consistent with the results of a study by Abdel - Daim et al. (1). However, they did not report any weight loss 48 hours after colitis; they measured initial and final weight and did not report the protective effects of spirulina on weight loss after colitis. In addition, their criteria for bleeding were different from ours, and they did not measure stool consistency and clinical activity of colitis.

Based on experimental research, flavonoids in honey (rutin and quercitrin) have antidiarrheal effects due to the improvement of colonic absorptive function and intestinal permeability, inhibition of muscle contractility, and a decrease of fluid accumulation in the gut and motility of intestine (13).

Previous studies have reported that kaempferol, a flavonoid in honey, has barrier integrity activities due to the inhibition of neutrophil migration and barrier disruption (29).

The current study showed that the administration of

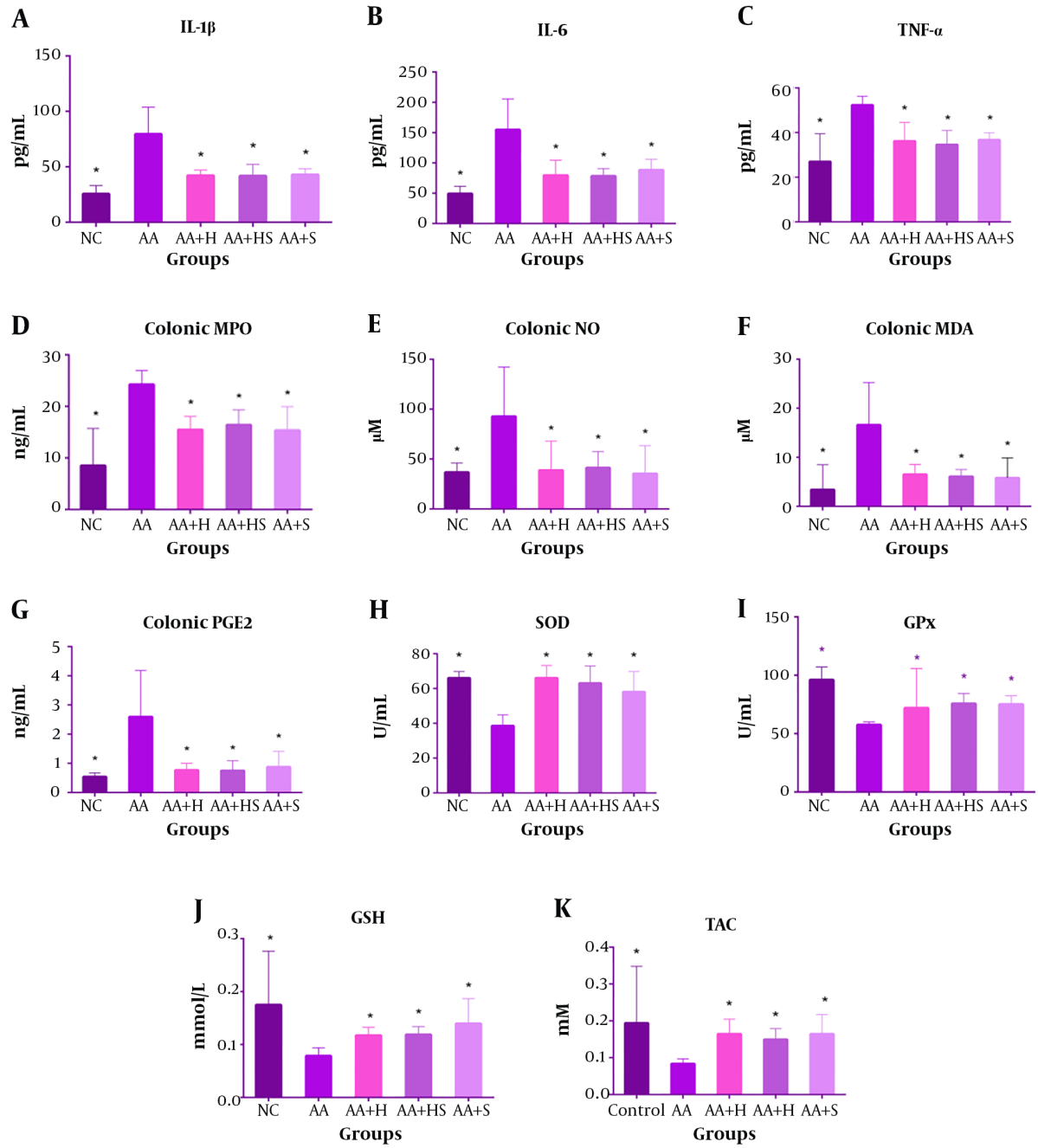

Figure 1. The effect of preadministration of honey, spirulina, and honey + spirulina on the biochemical parameters of rats with acetic acid-induced ulcerative colitis. Values expressed are median (IQR), N = 8. Values significantly different from the acetic acid-induced colitis group are shown by * ($P < 0.05$). NC = Normal Control, AA = Acetic Acid-induced colitis, H = Honey, HS = Honey + Spirulina, SP = *Spirulina Platensis* IL-6 = Interleukin, TNF = Tumor Necrosis Factor, NO = Nitric Oxide, MPO = Myeloperoxidase , MDA = Malondialdehyde, PGE2 = Prostaglandin E2 , SOD = Superoxide Dismutase ,GPx = Glutathione Peroxidase, GSH = reduced Glutathione, TAC = Total Antioxidant Capacity.

honey and SP individually and SP + honey attenuated neutrophil infiltration, as indicated by a significant decline in the colonic MPO activity as a quantitative and sensitive biomarker of neutrophil infiltration and inflammation in inflamed tissues (20). Previous investigations did not examine the effect of oral pre-administration of honey on MPO activity in colitis but honey - treated rats after the induction of colitis showed decreased MPO (5, 19). Chrysin,

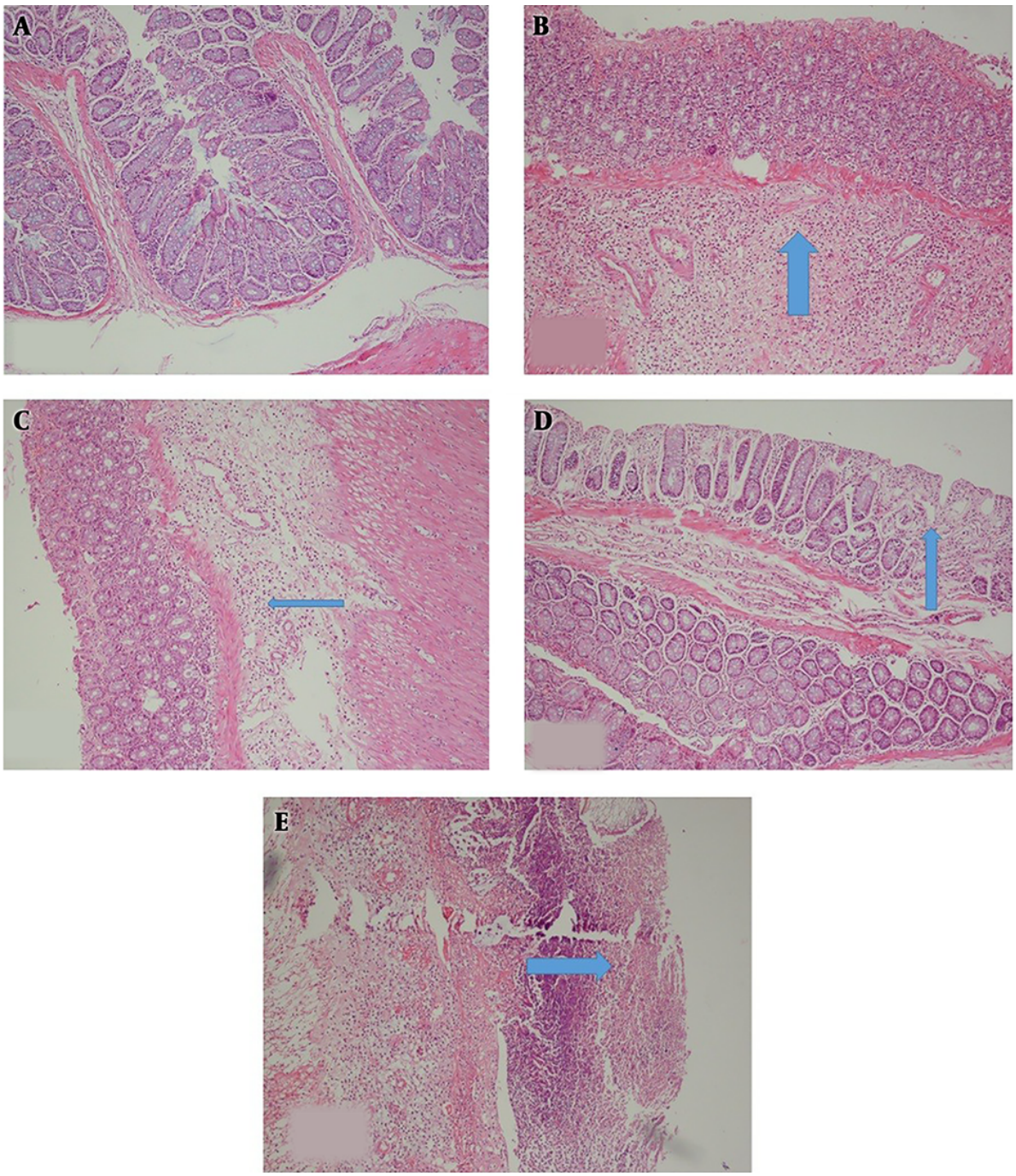

Figure 2. Photomicrographs of colonic sections, H&E (X 250). A: Normal colon mucosa of the normal control group. B and C: Mucosal ulceration with inflammation extending to submucosa in groups pretreated with honey and honey + spirulina, respectively, D: Pretreatment with spirulina showing mild mucosal inflammation and architectural gland disarray and crypt hyperplasia. E: Colonic mucosa with necrosis, ulceration, and inflammation extending to muscular layer in the acetic acid-induced colitis group. Arrows show damaged tissues.

a flavonoid in honey, can reduce the colonic MPO activity (30). In agreement with our study, Abdel - Daim et al. showed decreased colonic MPO by 15 days of SP pre-administration (500 mg/kg) in colitis rats (1). In a very

short period intervention, phycocyanin significantly reduced colonic MPO when administered 30 minutes before colitis induction (31).

The present investigation showed that the pretreatment of rats with SP and honey individually and in combination had protective effects against oxidative stress and lipid peroxidation, as presented by reduced colonic MDA, a marker of lipid peroxidation in tissues (10). They also attenuate inflammation as evidenced by a decrease in the NO levels. In line with our study, pretreated rats with SP for 15 days showed a decreased colonic MDA level and iNOS expression (1). In addition, the pre-administration of honey with the same dose as ours, but for four days, reduced the rat colonic MDA level (32). Spirulina contains a wide spectrum of antioxidants with synergistic activities in free radical scavenging. Phycocyanin, which its antioxidant potential is about 20 times higher than that of vitamin C, and phycocyanobilin can suppress oxidative stress through their radical scavenging activities and reduce the ROS production due to the inhibition of NADPH oxidase activity (33, 34). Overall, polyphenolic substances in honey have ROS and NO radical scavenging activities. For instance, polyphenols (e.g., caffeic acid, ellagic acid, chrysin, and quercetin) by down - regulation of NF-κB decreased the biosynthesis of iNOS and inhibited NO production (28, 35). Gallic acid, a phenolic compound in honey, can decline lipid peroxidation by free radical scavenging (36). Previous studies have reported that kaempferol, pinobanksin, and chrysin phenolic components in honey by repression of NF-kB signaling decreased the production of MDA and NO (29, 30, 37). NF-κB induces proinflammatory gene expression under oxidative stress and inflammatory conditions and increases cytokine production (e.g., interleukins, TNF-α, COX-2, and iNOS) (14).

We showed that the administration of SP and honey individually and SP + honey could reduce inflammation, as indicated by a substantial decline in PGE2, TNF-α, IL-6, and IL-1β level. We did not find any literature assessing the effects of oral honey preadministration on PGE2, IL-6, IL-1β, and TNF-α in colitis. Few studies have examined the therapeutic, but not protective, effects of oral honey in experimental colitis (18). The effects of pretreatment with oral spirulina on these inflammatory markers have been evaluated in only one study. In agreement with our findings, it was revealed that preadministration of SP for 15 days could decrease TNF-α, PGE2, IL-6, and IL-1β levels (1).

Phycocyanin selectively inhibits COX2 (38). It also inhibits NF-κB activation, modulates MAPK pathways (16), inhibits the production of TNF-α, suppresses COX-2 expression, and consequently decreases the production of PGE2 (39) and up-regulates the expression of PPARγ in colonic epithelial cells (40). Beta-carotene in spirulina blocks NF-κB activation consequently suppresses COX-2, TNF-α, and iNOS expression and inhibits the production of PGE2 and NO. In addition, β-carotene inhibits the generation of IL-1β and IL-6 in stimulated macrophages by suppressing their transcription (16, 39).

Additionally, ergothioneine exhibits anti-inflammatory, antioxidant, and cytoprotective properties. It inhibits the expression of IL-6, IL-8, and TNF-α and prevents cell death, caused by free fatty acids through the activation of JNK and p38MAPK signaling pathways (41). On the other hand, honey may be a selective COX-2 inhibitor (33). Hussein et al. indicated that Gelam honey could reduce plasma TNF-α, PGE2, IL-6, and NO levels by suppression of COX-2 and iNOS in carrageenan - induced acute paw edema in rats (42). These findings confirm anti-inflammatory properties of honey. It has been stated that polyphenols in honey exert their wound healing properties and a part of antioxidant effects through up-regulation of AMPK/ Nrf2/ARE signaling pathways and promote antioxidant enzyme expression (37). In addition, chrysin suppresses the expression of COX2 and release of IL-1β and TNF-α (43). Gallic acid can suppress iNOS and COX2 and diminish the production of proinflammatory cytokines and histamine release in macrophages (28). In addition, Kassim et al. reported that honey induced heme oxygenase-1, which inhibits NF-κB and iNOS and attenuated NO, IL-1β, and TNF-α generation in LPS-induced endotoxemia rats (44). COX2, IL-6, and TNF-α expression reduced in mice receiving chrysin before the induction of colitis (30).

Our findings showed that preadministration of SP and honey individually and concomitantly caused a significant improvement in the antioxidant defense system, indicated by restoration of TAC and GSH levels, besides GPX and SOD activities, in comparison with the AA-colitis group. We did not find any report evaluating the effects of oral honey preadministration on TAC level, as well as SOD and GPX activity, and the effects of oral pretreatment with SP on GPX and TAC levels in experimental colitis. Our findings are consistent with the results of a study by Abdel - Daim et al., which indicated an increase in SOD and GSH levels after preadministration of 500 mg/kg oral SP over 15 days in colitis rats (1). Mahgoub et al. showed that preadministration of 5 g/kg of honey for four days (both orally and intrarectally) led to the significant inhibition of colonic mucosal GSH depletion in colitis rats (32). However, their duration of intervention was decidedly shorter than what used in our study. A study reported that manuka honey keeps GSH in the reduced form and protects its activity; it also protects GPx and SOD activity and removes lipid hydroperoxides and hydrogen peroxide from the gastric mucosal cell of rats (45).

The first line of endogenous defense, which can prevent oxidative damage, includes enzymatic (SOD, GPX, and CAT) and nonenzymatic (GSH) antioxidants (46). Decreased antioxidant capacity contributes to the pathogenesis of UC and other inflammatory disorders (18). Spirulina and honey are excellent sources of powerful antioxidants; both contain antioxidant enzyme SOD, phenolic compounds, and carotenoids. In addition, honey contains GSH and catalase (18). Polyphenols exert a part of their antioxidant effects through promoting the secretion of antioxidant enzymes (e.g. SOD and CAT) and induction of HO-1 and GSH-linked detoxifying enzymes, which are involved in the detoxification of xenobiotics (37). In addition, honey contains antioxidant enzymes, such as catalase, GSH, and SOD (21). Therefore, the improvement of antioxidant status in this study can be attributed to honey and spirulina polyphenols.

Strengths of our study are as follows: (i) The length of the intervention period (one month is a good time for a long-term preventive intervention); (ii) We examined the effect of two natural foods that are a unique collection of all their bioactive substances in a safe dose, relatively inexpensive, and easily accessible to everyone. This is while many studies have reported the effects of the extracts of honey and spirulina, their polyphenols, and other antioxidant substances, which are expensive and not readily available to everyone; and, (iii) In our study, a significant number of pro-inflammatory cytokines, antioxidants, oxidative stress markers, in addition to histological changes, weight loss, colonic weight/length ratio, and TCS were measured simultaneously. Our study had a limitation; we did not evaluate intestinal microbiota and hormones because of financial constraints.

4.1. Conclusion

The present results showed that oral preadministration of honey and SP individually and in a combination regimen could protect against AA-induced colitis in rats owing to their anti-inflammatory and antioxidant effects. Honey and SP exerted their beneficial effects via restoration of the endogenous antioxidant defense system, down-regulation of various inflammatory pathways, and reduction of neutrophil infiltration.

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