

Investigation of protective effects of *Harpagophytum procumbens* extract in an experimental rat colitis model

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Summary

The aim of this study was to investigate the possible effects of *Harpagophytum procumbens* (HP) extract on pro-inflammatory cytokines, oxidative stress, apoptosis and antioxidant mechanisms in a rat colitis model induced by acetic acid. 40 Wistar albino rats were divided into five equal groups: (1) Control, (2) HP, (3) Colitis, (4) Colitis + HP, (5) Colitis + Sulfasalazine. Malondialdehyde (MDA), total antioxidant capacity (TAS), superoxide dismutase (SOD), nitric oxide (NO), interleukin-6 (IL-6), tumor necrosis factor- α (TNF- α), ceruloplasmin and sialic acid levels were analyzed in blood samples. In addition colon tissues were evaluated histopathologically and immunohistochemically (B-cell lymphoma 2 (Bcl-2) and Bcl-2 associated X protein (Bax)). A significant decrease in serum MDA, NO, TNF- α , IL-6, sialic acid levels, and an increase in serum SOD, TAS, ceruloplasmin levels were observed in Colitis + HP group compared to Colitis group. On the other hand, HP administration moderately reduced histopathological damage. It was detected immunohistochemically (Bcl-2, Bax) that apoptosis levels increased in the Colitis group and decreased with HP application. HP administration ameliorated the biochemical and pathological changes caused by colitis in rats. According to the results obtained in this study; HP might have a protective effect by suppressing of proinflammatory cytokines, apoptosis and reduced oxidative stress in colitis.

Keywords: antioxidant, apoptosis, *Harpagophytum procumbens*, proinflammatory cytokines, rat

Ulcerative colitis (UC) is a chronic idiopathic disease characterized by inflammation of the colon wall with ulcerative areas and bleeding, diarrhea, and abdominal pain (25). Numerous environmental, genetic, and immunological factors may contribute to the development of ulcerative colitis (17, 26). There are humoral and cellular factors, as well as immunological factors, which can cause inflammation in ulcerative colitis (18). Tissue damage occurs due to the activation of the immune cells, as well as the excessive release of cytokines and inflammatory mediators. All of these mechanisms result in inflammation (6). Cytokines are crucial molecules in the initiation, regulation, and immune response of inflammation. For normal colonic homeostasis, anti-inflammatory and pro-inflammatory cytokines must be in equilibrium in the colonic mucosa. This equilibrium is disrupted by immune hyperactivation in UC. There is an increase in proinflammatory

cytokines, such as tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6), and nitric oxide (NO) (15).

For many years, sulfasalazine has been used as the primary drug in the treatment of ulcerative colitis. Researchers have aimed to develop more effective and safer drugs because of the side effects of sulfasalazine (9). Herbal therapies have been shown to have fewer side effects in chronic inflammatory diseases, such as ulcerative colitis, but their effects on the molecular mechanisms of such diseases remain unexplained (3). It has been reported that herbal medicines used to treat osteoarthritis reduce lipid peroxidation and increase the level of antioxidant defense components, such as superoxide dismutase (SOD), and the total antioxidant status (TAS) (4). According to the most recent studies on the biological effects of *Harpagophytum procumbens* (HP) in humans and experimental animals, it has many traditional uses, including fever reduction, pain relief, joint

diseases, diabetes, and malaria treatment. According to clinical studies on HP, it has fewer side effects than pharmaceutical non-steroidal anti-inflammatory drugs (4, 12, 13). The major active components in the roots of HP are iridoid glycosides (1). Studies indicate that iridoid glycosides have significant anti-inflammatory activity (43).

It has been stated that the insufficient and persistent immune response against intestinal bacteria plays a significant role in the pathogenesis of ulcerative colitis. There is a heightened T cell response to intestinal contents and excessive neutrophil infiltration into the colon tissue (18). This event results in uncontrolled inflammation. Interleukin-1 β , IL-6, tumor necrosis factor (TNF)- α , IL-12 and interferon (IFN)- γ have all been shown to have an effect on mucosal inflammation in humans and animals. It has been reported that oxidative stress increases and antioxidant activity decreases in colitis models (41).

Oxidative stress is the formation of cellular damage within an organism as a result of the disruption of the mechanism between the oxidants and antioxidants in favor of oxidants, lipid peroxidation and the release of reactive oxygen products. If the body's defense systems (antioxidant mechanisms) are insufficient against oxidative stress, oxidative damage develops in the cells and functions are substantially disrupted (14).

Although reactive oxygen species are highly active chemical structures, they target macromolecules such as proteins, lipids and nucleic acids. They cause protein deformation, lipid peroxidation (end product malondialdehyde (MDA)) and DNA mutations in these molecules (39). Free radicals are another highly reactive species that can damage DNA, proteins, carbohydrates, lipids and cell membranes. It resists these oxidants with antioxidant enzyme systems such as catalase (CAT), and SOD, which have cytoplasmic, mitochondrial and extracellular forms in living organisms, as well as antioxidant defense systems such as ceruloplasmin and transferrin (38).

Apoptosis is considered a vital component of various processes including a normal cell cycle, functioning of the immune system and chemical induced cell death. Apoptosis is triggered by the breakdown of the antioxidant defense, also occurs as a defense mechanism such as in immune reactions or when cells are damaged by noxious agents or diseases. Apoptosis is the programmed destruction of cells that have lost their functions as a requirement of intercellular relations in complex organisms without harming the environment (19). Nuclear factor erythroid 2 (Nrf2) has been identified as an inhibitor of apoptosis. HP is anti-apoptotic through activation of Nrf2 signaling pathway.

In this study, we hypothesized that HP may have a potentially protective effect against colitis. We aimed to investigate possible protective effects of HP on acetic acid-induced colitis in rats by identifying inflammatory mediators (NO, TNF- α , IL-6), oxidative

stress (MDA), antioxidant biomarkers (SOD, TAS), histopathological changes and apoptosis (Bcl-2, Bax).

Material and methods

Animals. In this study, 40 healthy adult male Wistar Albino rats (8-16 weeks old, each 210-380 g) were used. The animals were obtained from Balikesir University Experimental Animal Production, Care, Application and Research Center (BAUN DEHAM). During the experimental period, rats were housed under standard laboratory conditions (12 hours light/12 hours dark, 22°C, 40-60% relative humidity, *ad libitum* feeding). All procedures and tests were performed according to the European Economic Community Guidelines for use of laboratory animals and were confirmed by the Balikesir University Animal Experiments Local Ethics Committee (Approval number: BAU-HADYEK 2020/1-4). This study funded is by the Scientific Research Projects Unit of Balikesir University, Turkey (grant no: 2021/008).

Experimental design. Rats were randomly divided into the five equal groups. Groups are divided as follows: 1) Control (2 ml, physiological saline (FTS), orally, 14 days. At the end of the 14th day, 2 ml, FTS, intrarectally); 2) HP (HP, 300 mg/kg, 2 ml, orally, 14 days); 3) Colitis (FTS, 2 ml, orally, 14 days. At the end of the 14th day, 2 ml, 3% acetic acid, intrarectally); 4) Colitis + HP (HP, 300 mg/kg, 2 ml, orally, 14 days. At the end of the 14th day, 2 ml, 3% acetic acid, intrarectally); and 5) Colitis + Sulfasalazine (Sulfasalazine, 100 mg/kg, gavage, 14 days. At the end of the 14th day, 2 ml, 3% acetic acid, intrarectally). The administration time and doses of the drugs were based on a previous study (22, 30).

Sample collection. Blood samples were collected 24 hours after the administration of acetic acid under isoflurane anesthesia (inhaling 3 Vol% isoflurane in a chamber for 3-5 min and maintained anesthesia at 1-1.5 Vol% isoflurane with 0.8 L/min air as the carrier via a nose cone). The samples were centrifuged at 2500 \times g for 15 minutes to separate the serums and stored at -80°C until further analysis. After the cervical dislocation procedure under isoflurane anesthesia, colon tissue samples were taken, washed with FTS and preserved in 10% formol. Then, tissue samples were used to create paraffin blocks for immunohistochemical and histopathological evaluation.

Biochemical analysis. MDA, TAS, SOD, TNF- α , IL 6, NO, ceruloplasmin and sialic acid levels were analyzed in serum samples.

The MDA value in the serum is based on the spectrophotometer (Shimadzu UV-1800, Japan) evaluation of the red-pink colored complex that reacts with thiobarbituric acid (TBA) and exhibits maximum absorbance at 535 nm wavelength. To detect lipid peroxidation (MDA) levels, the method of Yoshioka et al. (42) was used.

SOD enzyme activity were analyzed following the methods used by Sun et al. (33). This method is based on the inhibition of nitrobluetetrazolium (NBT) reduction of superoxide formed by the xanthine/xanthine oxidase enzyme system by SOD in the sample. As a result of superoxide radicals reducing NBT and form formazone, which gives the maximum absorbance at 560 nm.

TAS levels were measured from the serum samples using a commercially available kit (TAS kit, Rel assay diagnostics, Gaziantep, Turkey). A stable antioxidant known as Trolox Equivalent was accepted as the standard for TAS calculation.

NO (Bioassay Technology Laboratory, BT Lab, E0703Ra, Shanghai, China), TNF- α (Bioassay Technology Laboratory, BT Lab, E0764Ra, Shanghai, China) and IL-6 (Bioassay Technology Laboratory, BT Lab, E0135Ra, Shanghai, China) levels were measured using commercially available ELISA kits according to the manufacturer's protocol utilizing an ELISA reader (Multiskan FC Microplate Photometer, Thermo, Waltham, MA, USA).

Ceruloplasmin levels were measured using the Sunderman and Nomoto (34) method. According to the procedure, a different colored oxidation product is formed from the ceruloplasmin and the p-phenylenediamine component. The resultant correlates with serum ceruloplasmin values.

Sialic acid levels of the serums were measured at 549 nm by the method of Warren (40) in which sialic acid reacted with thiobarbituric acid to form a red product.

Histopathological examination. Colon samples were fixated in 10% formaldehyde solution for 72 h. After standard fixation and follow-up with alcohol and xylene solutions, the tissues were embedded in paraffin blocks. After 5 μ m thick serial sections were taken from the paraffin blocks in a microtome (Leica 2245, Nussloch, Germany). Hematoxylin and Eosin (H&E) staining technique was performed on certain sections. Each group's specimens were examined under a light microscope and photographed. Microscopic grading and statistical data of colon damage were evaluated using the method reported by Appleyard and Wallace (2). Loss of mucosal architecture (0-3), inflammatory cellular infiltration (0-3), crypt abscess formation (0-1), goblet cell depletion (0-1) are the criteria scores for this evaluation method.

Immunohistochemical evaluation. Tissue samples were fixated in formalin solution for 72 h. Tissue sections were blocked by passage through alcohol, xylol and were encased in paraffin blocks. After the follow-up and blocking procedures, 5 μ m sections were taken in poly-l-lysine coated slides. The sections were evaluated immunohistochemically using Avidin-Biotin peroxidase complex method (for Bcl-2 and Bax staining) according to the manufacturer's

recommendations. In immunohistochemical processes, endogenous peroxidase activity was inhibited and then incubated with primary antibody (Bcl-2 or Bax) (Anti-rat Bax antibody, dilution 1 : 200, Santa Cruz, CA, USA). Sections were incubated with secondary antibody (1 : 200; Santa Cruz Biotechnology, Inc., CA, USA) and subsequently incubated with streptavidin peroxidase complex. After washing with phosphate buffered saline (PBS), the slides were treated with diaminobenzidine. Sections were counterstained with hematoxylin and mounted. Finally, Bcl-2 and Bax positive cells were evaluated semi-quantitatively under the light microscope (Nikon, Eclipse Ci, Tokyo, Japan) and selected areas were photographed.

Statistical evaluation. In each group, all data were expressed as mean and standard error (as mean \pm SE). Statistical analysis of the differences between the groups was performed using ANOVA. Post hoc multiple comparisons were performed utilizing Duncan's test. If the obtained data were not normally distributed (Histopathological examination), non-parametric Kruskal-Wallis data analysis was applied for the comparative test. All analyses were performed using the SPSS (Version 17.0, Chicago, IL, USA) software program. The difference between the groups in terms of the parameters examined was considered statistically significant at the $P < 0.05$ level.

Results and discussion

Biochemical indices. The serum MDA values of the Colitis group shown in Table 1 were higher than the Control and HP groups. Serum MDA levels decreased significantly in the Colitis + HP group compared to the Colitis group ($P < 0.05$). However, serum SOD levels of the Colitis group were lower than those in the Control and HP groups ($P < 0.05$). As indicated in Table 1, administration of the HP extract in the Colitis group significantly increased the serum SOD values. The serum TAS levels were observed to be lower in the Colitis group than in the Control group. Serum TAS level increased with the application of the HP extract in the Colitis + HP group compared to Colitis group. As seen in Table 1, the levels of inflammatory biomarkers such as IL-6, TNF- α and NO were significantly higher in the Colitis group compared to the Control and HP

Tab. 1. Biochemical parameters of the serum samples and statistical results in each group (Mean \pm SE)

Parameters	Control (n = 8)	HP (n = 8)	Colitis (n = 8)	Colitis + HP (n = 8)	Colitis + Sulfasalazine (n = 8)
MDA (μ mol/L)	14.80 \pm 0.96 ^c	13.64 \pm 0.75 ^c	24.90 \pm 1.19 ^a	19.11 \pm 0.47 ^b	18.99 \pm 0.49 ^b
SOD (U/L)	60.87 \pm 1.14 ^a	61.46 \pm 0.93 ^a	50.64 \pm 0.50 ^c	54.13 \pm 0.56 ^b	56.40 \pm 0.74 ^b
TAS (mmolTrolox Equiv./L)	1.35 \pm 0.02 ^a	1.38 \pm 0.02 ^a	0.82 \pm 0.04 ^c	1.14 \pm 0.03 ^b	1.18 \pm 0.03 ^b
NO (μ mol/L)	26.50 \pm 1.16 ^c	27.44 \pm 1.11 ^c	40.74 \pm 1.31 ^a	33.15 \pm 0.67 ^b	32.95 \pm 0.68 ^b
TNF- α (ng/L)	41.19 \pm 0.56 ^c	40.92 \pm 1.31 ^c	73.60 \pm 1.29 ^a	56.62 \pm 1.60 ^b	54.75 \pm 1.74 ^b
IL-6 (pg/ml)	3.40 \pm 0.14 ^c	3.26 \pm 0.09 ^c	5.60 \pm 0.10 ^a	4.42 \pm 0.10 ^b	4.39 \pm 0.08 ^b
Ceruloplasmin (mg/dl)	40.67 \pm 1.16 ^a	40.16 \pm 0.47 ^a	23.80 \pm 0.95 ^c	30.16 \pm 0.94 ^b	31.78 \pm 0.93 ^b
Sialic acid (μ g/ml)	467.89 \pm 15.79 ^d	471.28 \pm 14.98 ^d	865.64 \pm 19.80 ^a	683.90 \pm 14.90 ^b	630.56 \pm 10.68 ^c

Explanations: a, b, c, d – the differences between groups in the same row including different letters are statistically significant ($P < 0.05$); HP – *Harpagophytum procumbens*; MDA – malondialdehyde; SOD – superoxide dismutase; TAS – total antioxidant status; NO – nitric oxide; TNF- α – tumor necrosis factor- α ; IL-6 – interleukin-6

groups. Administration of the HP extract significantly decreased the concentrations of these biomarkers in the Colitis group. Furthermore, ceruloplasmin values

were found to be significantly lower ($P < 0.05$) in the Colitis group compared to the Control and HP group. Administration of the HP extract significantly

Tab. 2. Histopathological results in colon tissue of the groups (Mean \pm SE) (H&E)

Parameters	Control (n = 8)	HP (n = 8)	Colitis (n = 8)	Colitis + HP (n = 8)	Colitis + Sulfasalazine (n = 8)	Asymp. Sig. Kruskal-Wallis test (P Value)
Loss of mucosal structure	0.12 ± 0.12^c	0.25 ± 0.16^c	2.62 ± 0.18^a	2.37 ± 0.18^a	1.75 ± 0.31^b	0.000
Inflammatory cell infiltration	0.50 ± 0.27^c	$1.00 \pm 0.38^{b,c}$	2.37 ± 0.18^a	2.00 ± 0.33^a	$1.50 \pm 0.27^{a,b}$	0.002
Crypt abscess formation, edema, congestion	0.00 ± 0.00^b	0.12 ± 0.12^b	0.87 ± 0.12^a	0.75 ± 0.16^a	0.62 ± 0.18^a	0.001
Goblet cell depletion	0.00 ± 0.00^b	0.00 ± 0.00^b	0.87 ± 0.12^a	0.75 ± 0.16^a	0.62 ± 0.18^a	0.000

Explanations: a, b, c – the differences between groups in the same row including different letters are statistically significant; HP – *Harpagophytum procumbens*

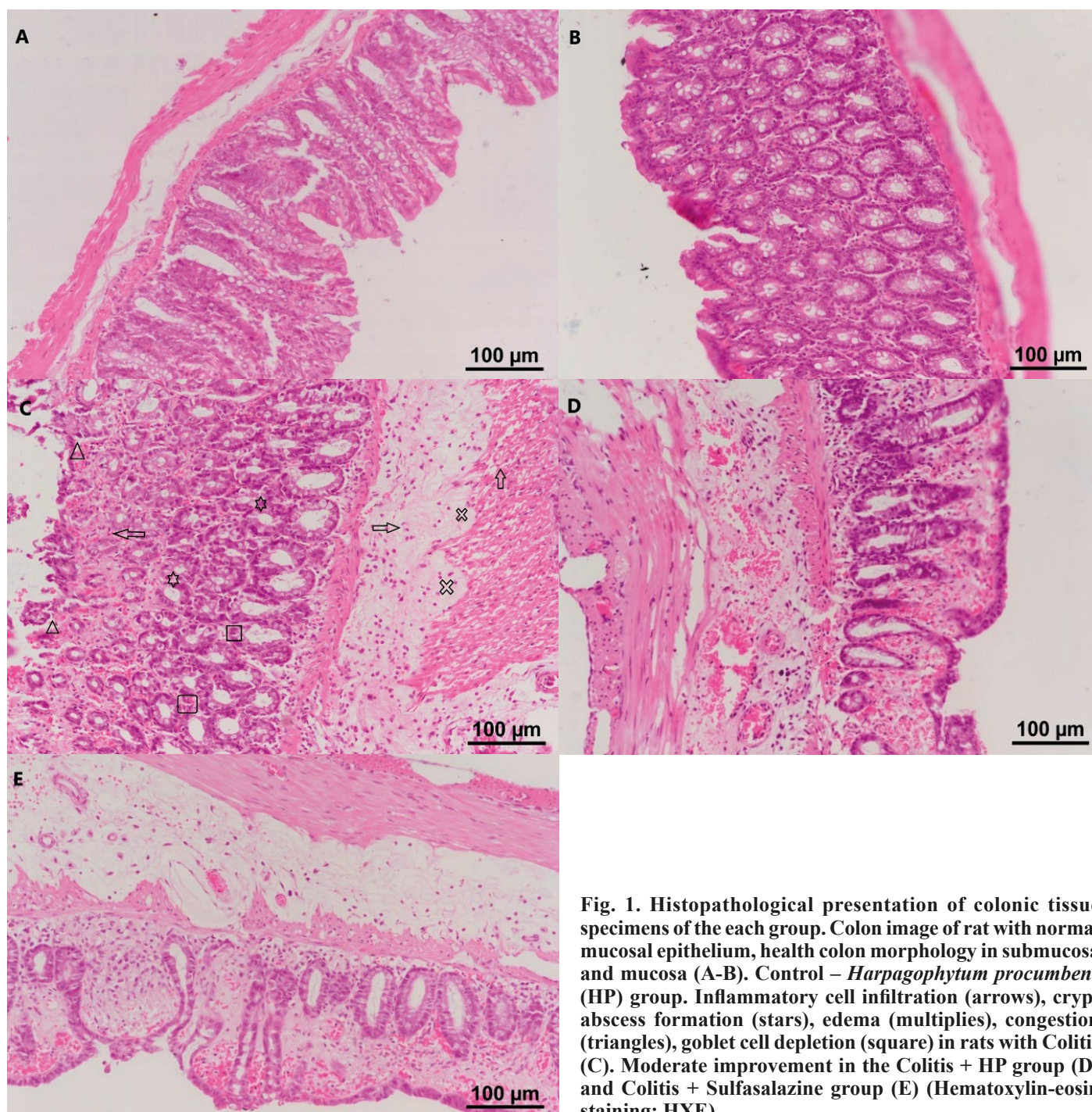


Fig. 1. Histopathological presentation of colonic tissue specimens of the each group. Colon image of rat with normal mucosal epithelium, health colon morphology in submucosa and mucosa (A-B). Control – *Harpagophytum procumbens* (HP) group. Inflammatory cell infiltration (arrows), crypt abscess formation (stars), edema (multiplies), congestion (triangles), goblet cell depletion (square) in rats with Colitis (C). Moderate improvement in the Colitis + HP group (D) and Colitis + Sulfasalazine group (E) (Hematoxylin-eosin staining: HXE)

increased the serum ceruloplasmin levels in the Colitis + HP group when compared to the Colitis group. As indicated in the table, sialic acid concentration in the Colitis group were significantly higher than those in the Control and HP groups ($P < 0.05$). Treatment with HP decreased this concentration in colitis. There weren't any significant differences between the Control and HP groups in terms of these parameters. All parameters in each group are shown in Table 1.

The biochemical results showed that HP could reduce oxidative stress, increase the activity of antioxidant enzyme and decrease the expression of inflammatory cytokines induced by colitis.

Histopathological evaluation. The histopathological findings obtained from the tissue sections of the rats used in this study were graded according to the values given in Table 2. Each group's histopathological changes are shown in Figure 1. The microscopic view of Control and HP group rats with normal colonic mucosa with intact histopathological epithelium is shown in Figures 1A-B. The colon tissues in rats of the Colitis group showed signs of inflammatory cell infiltration, crypt abscess formation, edema, congestion, Goblet cell depletion (Fig. 1C). These histopathological damages were lower in the Colitis + HP and Colitis + Sulfasalazine groups in comparison

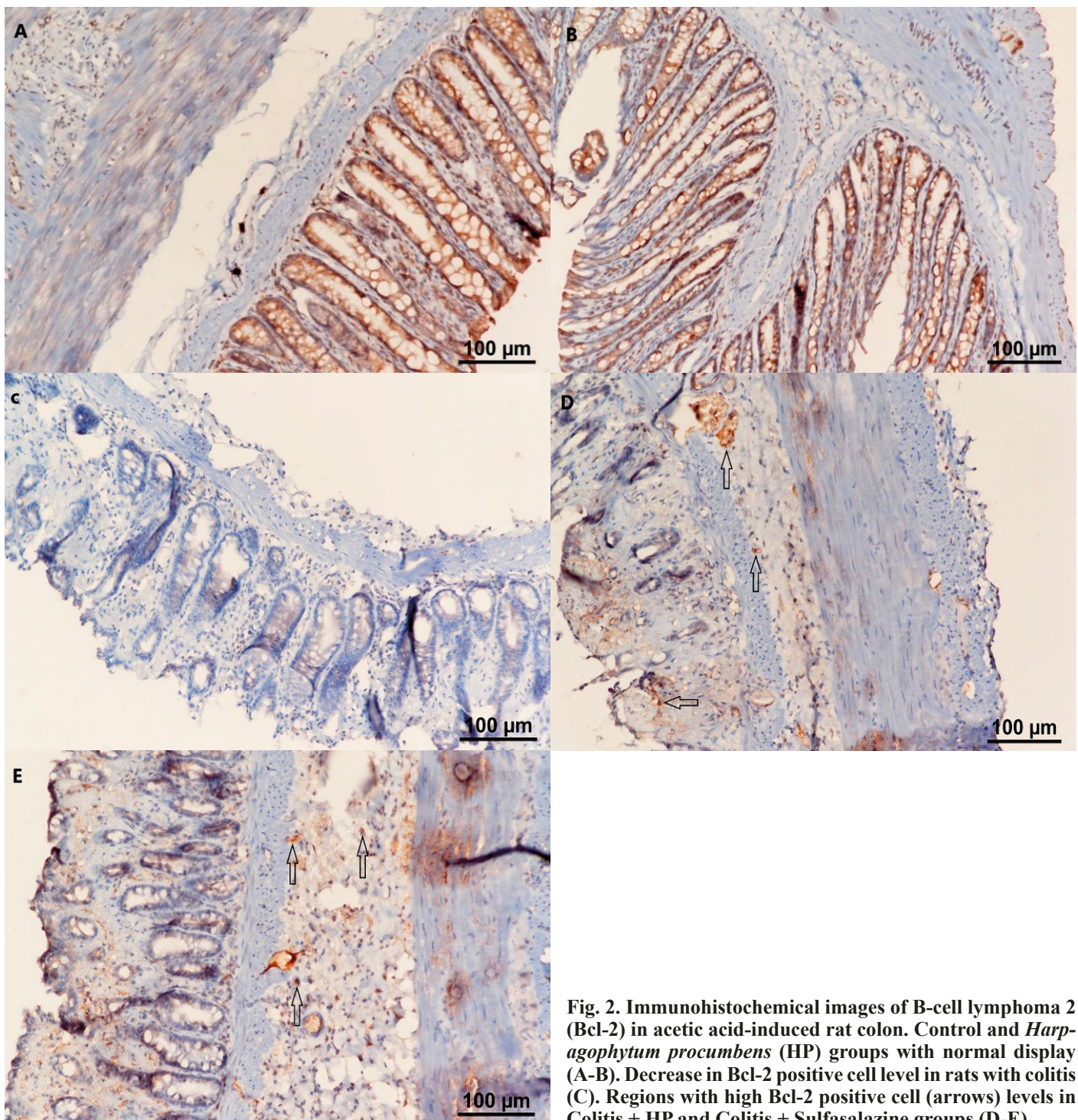


Fig. 2. Immunohistochemical images of B-cell lymphoma 2 (Bcl-2) in acetic acid-induced rat colon. Control and *Harpagophytum procumbens* (HP) groups with normal display (A-B). Decrease in Bcl-2 positive cell level in rats with colitis (C). Regions with high Bcl-2 positive cell (arrows) levels in Colitis + HP and Colitis + Sulfasalazine groups (D-E)

with the Colitis group (Fig. 1D-E). It was observed that the effect of extract application was moderate in these groups.

Immunohistochemical results. We investigated the effects of HP on the apoptosis rate in colitis by detecting Bax and Bcl-2 using the immunohistochemical method. Figure 2 depicts the microscopic image of Bcl-2 protein in each group. The number of Bcl-2 positive cells were higher in the Control and HP groups than in the Colitis group. No significant difference was detected in Bcl-2 positive cells in the Control (Fig. 2A) and HP (Fig. 2B) groups. A weak Bcl-2 im-

munoreactivity was seen in the Colitis group. However, the administration of HP increased the expression of Bcl-2. As seen in Figure 2C, there was a decrease in the number of Bcl-2 positive cells in the Colitis group. An increase in Bcl-2 positive cells was observed in the Colitis + HP (Fig. 2D) and Colitis + Sulfasalazine (Fig. 2E) groups compared to the Colitis group.

There was no difference in the number of Bax positive cells in the colon tissue samples in the Control and HP-treated groups (Fig. 3A and 3B), and a weak immunoreactivity was observed. However, the number of Bax positive cells increased in the Colitis group

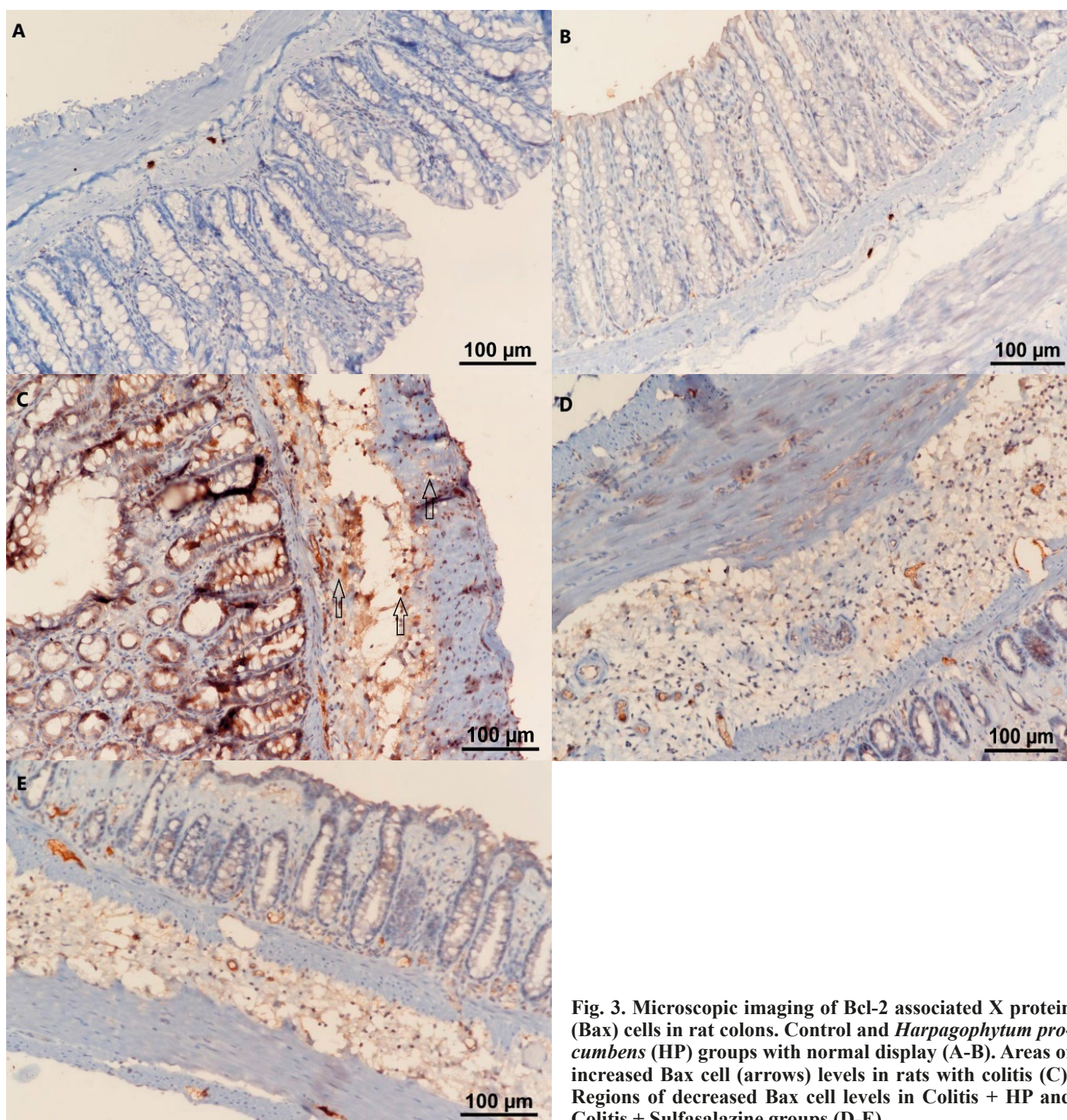


Fig. 3. Microscopic imaging of Bcl-2 associated X protein (Bax) cells in rat colons. Control and *Harpagophytum procumbens* (HP) groups with normal display (A-B). Areas of increased Bax cell (arrows) levels in rats with colitis (C). Regions of decreased Bax cell levels in Colitis + HP and Colitis + Sulfasalazine groups (D-E)

(Fig. 3C). On the other hand, it was observed that the number of Bax positive cells decreased in the Colitis + HP (Fig. 3D) and Colitis + Sulfasalazine groups (Fig. 3E), compared to the Colitis group. Inducing colitis in rats increased the number of apoptotic cells but using HP in rats with colitis reduced the number of apoptotic cells. In this study, antiapoptotic, anti-inflammatory and antioxidant effects were analyzed to demonstrate the potential protective role of HP in colitis. This is the first study concerning the protective effect of HP on colitis.

Ulcerative colitis is an inflammatory disease of the large intestine, which particularly affects the mucosa and submucosa. Although the causes of the disease are not fully known, genetic factors affect decreased antioxidant capacity. It is known that neutrophils and macrophages can cause colon damage by disrupting epithelial integrity in the disease (35).

In ulcerative colitis, inflammatory mediators are intensely secreted from granulocytes that migrate to the damaged mucosa (44). Oxidative stress is one of the key factors in the etiopathogenesis of the disease. Antioxidant capacity is reduced in colitis due to free radical damage caused by lipid peroxidation, leading to excessive colonic inflammation (5).

TNF- α is a potent proinflammatory cytokine produced in response to chronic inflammation or tissue damage (11). It has been reported that IL-6, a proinflammatory cytokine directly induced in acute inflammation, is elevated in ulcerative colitis and has an important role in the pathogenesis of the disease (21). Since oxidative stress and inflammatory response play a significant role in the development of ulcerative colitis, there has been increased interest in natural products with antioxidant properties in recent years. In the study, proinflammatory cytokines such as IL-6 and TNF- α , NO, and MDA, a lipid peroxidation marker, were found to be significantly higher in the Colitis group. The findings obtained in the study showed that HP administration has a positive effect on the treatment of Colitis in rats by reducing oxidative damage in the blood. The results of this study are compatible with the scientific data revealed in some studies conducted in the past years (31, 32).

Harpagoside may exert an anti-inflammatory effect by inhibiting nuclear factor-kappa B and inhibiting LPS-induced iNOS and COX-2 expression (16, 20). This mechanism is believed to be partially responsible for the analgesic and cartilage-protecting effect of *H. procumbens* through inhibition of inflammatory mediators such as COX-2, leukotrienes, TNF- α and interleukin-1 (IL-1) (7). It has been reported that HP exerts its chondroprotective effect by inhibiting inflammatory mediators such as COX-2, NO, TNF- α , IL-1 β , as well as inhibiting matrix metalloproteinase (MMP) and elastase enzymes, which play a key role in cartilage degradation (8).

In a study performed on the mesangial cells of rats, it was determined that harpagoside inhibited the release of nitrite and iNOS, resulting in an anti-inflammatory effect. Studies have shown that *H. procumbens* directly inhibits proinflammatory cytokines (24). In mice with formalin-induced pain, *H. procumbens* extract reduced the duration of pain in a dose-dependent manner. The significant increase in nitrite/nitrate content in rat spinal cords caused by the formalin injection was significantly reduced by the *H. procumbens* extract (36). Parenti et al. (27) found that *H. procumbens* extract significantly reduced carrageenan-induced pain in rats with intraperitoneal administration.

Ucuncu et al. (37) administered *H. procumbens* extract containing 3% harpagoside orally to animals for 30 days in an experimental rheumatoid arthritis animal model. This prevented cartilage destruction. Examining the pharmacological effect relationship revealed that it suppressed oxidative stress and inflammation by decreasing the levels of malondialdehyde (MDA), nitric oxide (NO), 8-hydroxyguanine levels and proinflammatory cytokine levels.

The first defense process against free radicals in the body occurs with the enzyme SOD. SOD is an endogenously produced antioxidant that is necessary for every cell that makes up the organism. It is reported that SOD protects the organism from the damage of oxidants with its transforming effect on superoxide radical, which prevents the formation of peroxynitrite (14). In our study, administration of AA to the colon significantly decreased the values of antioxidants such as SOD and TAS. Our study demonstrated that the application of HP can significantly increase antioxidant levels. As a result, the increased antioxidant level had a positive effect on the colon. The histopathological evaluation of the study revealed that alterations such as loss of mucosal structure, inflammatory cell infiltration, crypt abscess formation, edema, congestion and goblet cell depletion in the colitis group slightly regenerated after being administered HP, which is in line with the result of the previous study. Previous studies have reported that the use of various antioxidant drugs have shown a decrease in histopathological findings (23, 28, 37).

Apoptosis is a form of morphologically programmed cell death that plays a crucial role in the elimination of some tissues in the body and some immunological cells that react with each other. The results demonstrated that HP administration significantly reduced proinflammatory cytokines, as well as decreased apoptosis by increasing the Bcl-2 (B-cell lymphoma 2) gene expression level and decreasing the Bax (Bcl-2 associated X protein) gene expression level in colon tissue, which is in line with literature (29).

The effects of HP have been demonstrated in several different disease models. In this study the anti-inflammatory, antioxidant and antiapoptotic effects were studied in colitis. But the molecular mechanisms

by which HP protects against acetic acid induced colon damage still remains unclear. Further study on the pharmacokinetics, protective molecular mechanisms and colon penetration of HP can explain its effects on colitis and provide evidence for future clinical applications. In this study, both microscopic findings and biochemical results confirm the occurrence of damage in the colitis model, with results showing that treatment of HP reverses this destruction.

In conclusion, the parameters for histopathological disorders, oxidative damage and inflammation increased in the colitis model studied in this research. However, the therapeutic effects of HP treatment on the parameters give rise to the belief that this antioxidant drug is effective in the treatment of colitis. The present study demonstrated that HP improved colitis induced injuries in the colon mucosa and submucosa through its antiapoptotic, anti-inflammatory and antioxidant effects. Therefore, HP may have a therapeutic potential against colitis. The mechanism of these effects is via the reduction of oxidative stress, apoptosis and inflammation, increasing the total tissue antioxidant capacity or possibly other unknown pathways, which requires further research studies. We suggest that HP may be used to protect against colitis, but additional studies are needed to confirm this assumption, and this will be investigated in future studies. Plants used in traditional medicine such as HP are seeing increasing use day by day due to their low side effects and clinical and biochemical benefits. The search for effective herbal supplements as a complementary treatment for ulcerative colitis is a complex issue, and due to the complexity of the study and protocols, there are not enough studies at present. For this purpose, longer-term studies should be conducted. There is a need for longer-term clinical studies on HP preparations at different doses. The mechanism of action of this extract needs to be further investigated and explained in more detail before this finding can be translated into clinical practice.

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