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RESEARCH

Mespilus germanica L. Fruit Extract Migitates Experimental Colitis by Modulating Inflammation and Apoptosis in Rats

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Abstract
Ulcerative colitis is an inflammatory bowel disease that affects the rectum and colon. Considering side effects of current medications and patient population unresponsive to these drugs, maintaining disease remission with novel natural dietary compounds appears to be a critical therapeutic strategy. This study aimed to investigate antioxidant, anti-inflammatory, anti-apoptotic effects of *Mespilus germanica* (MG) fruit in an acetic acid-induced colitis model in rats for the first time. Forty-eight male Wistar albino rats divided into six groups ($n=8$): control (saline), acetic acid (AA)+saline, AA+100 mg/kg/day MG, AA+300 mg/kg/day MG, AA+1000 mg/kg/day MG, and AA+100 mg/kg/day sulfasalazine. All treatments were administered by oral gavage over three days. Colonic tissue samples underwent macroscopic, biochemical, and histopathological analysis. Myeloperoxidase, lipid peroxidation, superoxide dismutase, and glutathione levels indicated that MG fruit extract lacked antioxidant properties. These findings were consistent with the extract's low in vitro antioxidant activity. Levels of Na/K-ATPase, Toll-like receptor-9, matrix metalloproteinase-3, caspase-3 and −9 were elevated in colitis group compared to control and decreased in MG groups. These findings support the notion that MG may contribute to the preservation of epithelial integrity through modulation of apoptosis-related pathways. TNF-α, IL-1β, and IL-17, which were higher in AA group than the control, showed significant reductions in MG groups compared to colitis group. MG reduce inflammation in rats with ulcerative colitis. AA-induced ulcerative colitis in rats was associated with edema, mucosal ulceration, the infiltration of inflammatory cells, a substantial increase in ulcer index and loss of mucosal glandular crypts. All treatments significantly reduced macroscopic and microscopic ulceration scores in colitis group. Current results suggest that MG treatment has potential to reduce disease activity by inhibiting inflammation and apoptosis and improving histological structure of colonic tissues.

Keywords Ulcerative Colitis · *Mespilus germanica* · Sulfasalazine · Macroscopic Scoring · Histopathology · Wistar Albino

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1 Introduction

Inflammatory bowel disease (IBD) is a chronic, immune-mediated condition that affects the gastrointestinal system. IBD comprises two subtypes: ulcerative colitis (UC) and Crohn's disease [1]. The pathogenesis of UC is multifactorial, involving genetic predisposition, defects in the epithelial barrier, dysregulated immune responses, and environmental factors [2]. UC is characterized by inflammation and ulceration of the colonic lining, impacting both the colon and rectum. It may result in various symptoms, such as abdominal pain, diarrhea, rectal bleeding, and weight loss. While there is no definitive cure for UC, various treatment options exist to manage symptoms and mitigate complications [3]. Depending on the severity of colitis, current treatment options include corticosteroids, antibiotics, aminosalicylates, immunosuppressants, and biological agents. However, these have been shown to have various side effects and are associated with resistance with long-term use. Therefore, the use of new approaches based on herbal treatments/natural products has been steadily increasing worldwide in recent years [4–10].

The pathogenesis of UC involves a complex interplay of genetic, environmental, and immune system factors. Certain genetic variants increase the likelihood of developing UC. These genetic variants may influence immune system activity or the functionality of the intestinal barrier [11]. Environmental factors, including smoking, dietary habits, and infections, may trigger ulcerative colitis in individuals with a genetic predisposition [11]. These stimuli activate intestinal immune responses, resulting in increased production of proinflammatory cytokines such as tumor necrosis factor- α (TNF-α), interleukin (IL)-1, IL-6, and IL-17 [11]. Evidence indicated that TNF-α, elevated during inflammation, contributes to intestinal damage primarily by enhancing matrix metalloproteinase (MMP) production in intestinal myofibroblasts. This process could result in the degradation of the extracellular matrix, tissue destruction, and ulcer formation [12].

The balance between inflammatory and anti-inflammatory cytokines is altered in UC. IL-1β is a key proinflammatory cytokine involved in inflammatory and apoptotic processes in UC [11, 13]. IL-17 also contributes to the amplification of intestinal inflammation [11, 12, 14]. IL-10 is a major anti-inflammatory cytokine regulating immune responses [15]. IL-10 plays a crucial role in preserving intestinal microbe-immune homeostasis; its deficiency leads to the onset of IBD due to an excessive immune reaction to intestinal microbiota [11, 12, 15]. Toll-like receptors (TLRs) are integral to host cell recognition and the immune response to microbial pathogens [16]. Activation of TLR signaling promotes the production of proinflammatory cytokines such as IL-1, IL-6, and TNF-α and enhances adaptive immune responses [17]. In IBD, T cells enhance the secretion of TNF-α and IL-17 cytokines and contribute to increased apoptosis, and are implicated in mucosal damage. The caspase activation cascade facilitates the rapid amplification of the apoptotic signal [18, 19]. Homeostasis of intestinal epithelial cells is maintained through regulated apoptosis within the crypt structure [20]. Caspase-9 levels in UC might vary based on the disease's pathophysiological status. It is involved in both extrinsic and intrinsic apoptosis pathways [18–20]. Myeloperoxidase (MPO) activity is widely used as a marker of neutrophil infiltration and colonic inflammation in UC [21]. Cellular homeostasis is also disrupted in IBD, and Na^+/K^+-ATPase has been associated with the regulation of apoptosis and autophagy [22]. MMP, particularly MMP-3, contribute to extracellular matrix degradation and tissue damage in IBD and may enhance apoptosis-related injury [23, 24].

Acetic acid (AA)–induced colonic injury initiates epithelial metabolic stress characterized by excessive reactive oxygen species (ROS) production and mitochondrial dysfunction. Elevated ROS levels activate the NF-κB signaling pathway via IκBα degradation, leading to transcriptional upregulation of pro-inflammatory cytokines including TNF-α, IL-1β, and IL-6. These cytokines amplify neutrophil infiltration and further oxidative burst, establishing a self-propagating inflammatory loop. Concurrently, TNF-α–mediated extrinsic apoptotic signaling and ROS-driven mitochondrial permeability transition increase the Bax/Bcl-2 ratio and activate caspase-3, resulting in epithelial apoptosis and crypt loss. Loss of epithelial integrity disrupts tight junction proteins (ZO-1, occludin, claudin-1), causing barrier dysfunction and enhanced luminal antigen translocation. Microbial products such as LPS activate TLR4-dependent NF-κB signaling, reinforcing the inflammatory cascade. Thus, oxidative stress–driven NF-κB activation represents the central mechanistic axis linking cytokine imbalance, apoptosis, and epithelial barrier failure in acetic acid–induced experimental colitis.

Mespilus germanica L., is a member of the subfamily Maloideae, includes two widely distributed species: *M. germanica* L. (common medlar) and *M. canescens* J.B. Phipps (Stern's medlar). Among these *Mespilus germanica* (MG), is the most prevalent species, native to Southeast Asia and has been recognized for centuries. Although referred to by its Latin name, German, the medlar is primarily located in the northern Caspian Sea region, Iran, and along the Black Sea coast of Türkiye [25]. The fruit of MG serves as a valuable nutritional resource and possesses multiple traditional medicinal applications [26]. The fruits of this species are used in traditional folk medicine in Türkiye, especially against intestinal inflammation [27]. Traditionally, it has

been utilized through various methods to address a range of diseases and symptoms, including dysentery, kidney and liver disorders, constipation, kidney and bladder stones, stomach pain, vomiting, stomach ulcers, colds, influenza, bronchitis, asthma, bladder infections, fever, and pain [26–32]. MG contains a variety of phytochemicals, including organic acids (tartaric acid, ascorbic acid, and oxalic acid), fatty acids (oleic acid, linoleic acid, and arachidic acid), amino acids (aspartate and glutamate), phenolic acids (chlorogenic acid, caffeic acid, and gallic acid), flavonoids (quercetin and rutin), coumarins (aesculetin), sugars (fructose, glucose, and sucrose), minerals (sulfur, barium, and sodium), and monoterpenes (phellandrene, γ-terpinene, and terpinolene). The plant has demonstrated antioxidant, antimicrobial, antidiabetic, and neuroprotective activities [33]. This study aimed to investigate, for the first time, the anti-inflammatory, antioxidant, and antiapoptotic effects of MG fruit extract, traditionally used for the treatment of intestinal inflammation, in an AA-induced UC model in experimental animals using phytochemical, biochemical, and histopathological analyses.

2 Materials and Methods

2.1 Experimental Animals

Forty-eight male Wistar Albino rats, weighing 200–250 g, used for this study were obtained from the Marmara University Experimental Animals Research and Implementation Centre in Istanbul, Turkiye. Rats were provided standard rat chow. Twelve hours prior to the induction of colitis, the rats were subjected to fasting in six wire cages, with access only to water. The animals were kept under conditions of 12-hour light/dark cycles, with a controlled temperature ranging from 22 to 24 °C and humidity levels between 45 and 65%. All experimental procedures adhered to the National Institute of Health guidelines for the care and use of laboratory animals and received approval from the Marmara University Local Ethics Committee for Animal Experiments (Approval no: 28.2023mar).

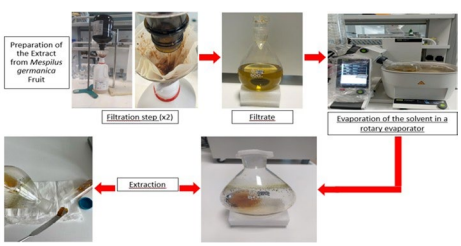


Fig. 1 Preparation steps of the extract from *Mespilus germanica* fruit

2.2 Preparation of *Mespilus germanica* (MG) Extract

The fruits of MG were purchased from a local boutique market in Kocaeli, Turkiye. The plant species was identified based on its fruits at the Department of Pharmaceutical Botany, Marmara University, by Dr. İsmail Senkardes. Fruit samples of the plant were documented at the Marmara University Faculty of Pharmacy Herbarium (MARE) with the identification number 22,986. The weight of the fresh ripe fruit of the plant was recorded at 1691.3 g. The material was separated from its seeds and subsequently weighed 1303 g after being processed into small pieces using a shredder. Fruit samples were immersed in 1400 mL of 90% ethanol for 48 h via the maceration method, followed by filtration through filter paper. This process was conducted two times (Fig. 1). All extracts obtained from the filtrations were combined, and the solvent was removed to dryness using a rotary evaporator to yield an aqueous ethanol extract with a yield of 13.87%, which was used in all analyses. Experimental doses of 100, 300, and 1000 mg/kg were prepared from this extract for the animal treatment groups, after which it was dissolved in distilled water using an ultrasonic bath and administered to the animals via orogastric gavage (OG).

2.3 In Vivo Experimental Design

Experimental animals were assigned randomly to six groups, each with eight animals. The groups were organized as follows: control group (normal saline-NS- group), AA+NS group, AA+100 mg/kg/day MG group, AA+300 mg/kg/day MG group, AA+1000 mg/kg/day MG group, and AA+100 mg/kg/day sulfasalazine group. On the first day of the experiment, the animals were fasted for 12 h. UC was induced on the second day under ether anesthesia by a single intrarectal (IR) administration of 1 ml of 5% AA solution (pH: 3), delivered via a polyethylene catheter inserted 8 cm proximally from the anus. Treatment was started simultaneously with the colitis induction AA administration. The treatment groups were given MG extract at doses of 100, 300 and 1000 mg/kg and sulfasalazine at dose of 100 mg/kg once daily for three days via OG. The control group received 1 ml of NS via a polyethylene catheter inserted 8 cm proximally from the anus under diethyl ether anesthesia (2 cc, inhalation), followed by daily administration of NS at a dose of 1 ml/kg/day via oral gavage for three consecutive days. All rats were decapitated under ether anesthesia one day after their last treatment. Sulfasalazine is one of the most commonly used first-line treatments recommended for both remission induction and maintenance therapy in mild to moderate UC. For this purpose, it is used as a reference drug at a dose of 100 mg/kg/day in experimental colitis studies [34].

3 Macroscopic Analysis

Following decapitation, the colons were excised beginning from the rectum, and an 8 cm segment was collected. The tissues were first rinsed with phosphate-buffered saline (PBS) and then weighed. Macroscopic scoring of the colons was performed, and photographs were taken. To minimize observer bias, all macroscopic assessments were performed by a researcher unaware of the experimental groups. The criteria presented in Table 1 were used to assess the degree of macroscopic damage induced by colitis [35]. Colon tissue samples were obtained from the most representative area for macroscopic scoring and subsequently used for light microscopic examination. The remaining portions were divided into equal parts for biochemical analyses and stored at −80 °C for homogenization.

4 Histopathological Analysis

The colon tissue samples from all experimental groups were fixed in 10% neutral buffered formalin at room temperature. The tissues were then dehydrated through a graded series of ethanol concentrations (70%, 90%, 96%, and 100%), cleared with xylene, soaked in liquid paraffin, and subsequently embedded in paraffin. Sections of 5 µm thickness were obtained from the paraffin blocks using a rotary microtome (Leica RM2125 RTS), mounted on slides. The tissue sections were stained with hematoxylin and eosin (H&E) and mounted with entellan for histopathological examination. Morphological changes in the colon tissues were evaluated using a photolight microscope (Olympus BX51, Tokyo, Japan) equipped with a CCD camera (Olympus DP72, Tokyo, Japan), and representative images were acquired from the sections. Histopathological evaluations and scoring were performed by an investigator blinded to the experimental groups to minimize observer bias. Tissue degeneration was semi-quantitatively scored in all samples at 20X magnification in one central and five peripheral areas based on the following parameters: (a) mucosal damage (surface epithelium and glands), (b) mucosal hemorrhage, (c) interstitial edema, and (d) inflammatory cell infiltration. Each parameter was graded on a scale of 0 to 3; 0: no damage, 1: mild, 2: moderate, and 3: severe [36]. The mean score for each parameter was calculated for each group, and the results were statistically analyzed.

Table 1 Macroscopic scoring criteria for ulcerative colitis

0	No damage
1	Focal hyperemia is present, no ulceration observed
2	Linear ulceration is present without hyperemia or thickening of the colonic wall
3	Linear ulceration accompanied by inflammation is observed in one region
4	Ulceration and areas of inflammation are present in two or more regions
5	Two or more major ulcerations and inflammatory areas, with damage in one or more locations extending over more than 1 cm of the colon
6–10	The area of ulceration and inflammation involves more than 2 cm of colonic length (the score is increased by one unit for each additional centimeter of damage).

5 Biochemical Analysis

In homogenized colon tissue samples, measurements of Na^+/K^+-ATPase (Cat. No. E1619Ra) MMP-3 (Cat. No. E0316Ra), Caspase-3 (Cat. No. E1648Ra), Caspase-9 (Cat. No. E1898Ra), TLR-9 (Cat. No. E0082Ra), MnSOD (Cat. No. E1376Ra), TNF-α (Cat. No. E0764Ra), IL-1β (Cat. No. E0119Ra), IL-17 (Cat. No. E0116Ra), and IL-10 (Cat. No. E0108Ra) were determined using commercial ELISA kits, following the protocol provided by the kit manufacturer (Bioassay Technology Laboratory). Additionally, myeloperoxidase (MPO) activity [37], lipid peroxidation (LP) [38], and glutathione (GSH) [39] measurements were performed as previously described. For MPO, the reaction was initiated after the addition of H_2O_2. After a 3-minute reaction time, the reaction was stopped by adding 30 µl of sodium azide. The tubes were centrifuged at 4 °C and 12.000 rpm for 1 min, and the absorbance was measured at 460 nm. The calculation was performed as: Absorbance × 204.6 units/g/min. For lipid peroxidation detection, the color formed after the reaction with thiobarbituric acid was measured spectrophotometrically at 535 nm. The absorbance value was multiplied by 128. For GSH, the color formed was measured spectrophotometrically at 412 nm, and the absorbance value was multiplied by 4. All biochemical assays were performed with a replicate number of 2.

5.1 In Vitro Anti-oxidant, Anti-inflammatory Analysis of MG Extract

The antioxidant activity of the extract was evaluated according to the method described by Zou et al. [40]. The total antioxidant capacity of the extract was determined by its radical scavenging activity against the ABTS [40]. The anti-inflammatory activity method (anti-lipoxygenase activity) described by Phosrithong et al. was adapted to a 96-well microplate format [41]. The phytochemical evaluation of the extract was conducted by determining the total phenolic content [42], total flavonoid content [43], and total triterpene content [44].

5.2 Statistical Analysis

The normality of data distribution was evaluated using the Kolmogorov–Smirnov and Shapiro–Wilk tests, complemented by visual inspection of histograms and Q–Q plots. Data meeting the assumptions of normality and homogeneity of variances were analyzed using Student's t-test for comparisons between two groups and one-way ANOVA for comparisons involving more than two groups. For data that did not meet parametric assumptions, non-parametric tests were applied, including the Mann–Whitney U test for two-group comparisons and the Kruskal–Wallis test for multiple-group comparisons.

When a significant overall difference was identified by ANOVA, post hoc analyses were performed using Tukey's multiple comparison test. Similarly, following a significant Kruskal–Wallis test, pairwise comparisons were conducted using Dunn's test with Bonferroni adjustment. A two-sided p-value < 0.05 was considered statistically significant. All statistical analyses were carried out using GraphPad Prism version 10 (GraphPad Software, San Diego, CA, USA).

6 Results

6.1 Macroscopic Analysis

The intestinal weights of the colitis (AA) group were significantly higher than those of the control group ($p = 0.0036$). Elevated intestinal weight was also elevated in the 100 mg MG, 300 mg MG, and sulfasalazine groups compared to the control group ($p = 0.019$), whereas the 1000 mg MG group did not differ significantly. None of the treatment groups showed a significant decrease compared to the colitis group (Fig. 2b).

Macroscopic evaluation showed significantly higher scores in the AA and 100 mg MG groups compared to the control group ($p < 0.001$ and $p = 0.011$, respectively). However, scores in the 300 mg MG, 1000 mg MG, or sulfasalazine groups were comparable to the control. Compared with the colitis group, macroscopic damage was significantly attenuated in the 300 mg and 1000 mg MG groups ($p = 0.014$ and $p < 0.01$, respectively) (Fig. 2a, c).

7 Histopathology

Light microscopic examination of colon sections from the control group revealed an intact mucosal structure, including epithelium, glands, lamina propria, and underlying submucosa (Fig. 3a). The colitis (AA) group exhibited mucosal damage with epithelial shedding, glandular loss, and necrosis, with prominent hemorrhagic foci in the lamina propria, accompanied by marked submucosal edema and inflammatory cell infiltration (Fig. 3b). The mucosal and submucosal damage noted in the 100 mg/kg MG group was largely similar to that of the colitis group (Fig. 3c). The 300 mg/kg MG group demonstrated partial mucosal regeneration marked by restoration of surface and glandular epithelium; however, localized epithelial loss, hemorrhage, and moderate submucosal inflammation remained (Fig. 3d). In the 1000 mg/kg MG group, mucosal regeneration was more pronounced, with reduced tissue damage and mild residual hemorrhage and inflammation (Fig. 3e). The sulfasalazine-treated group showed notable improvement in mucosa along with epithelial regeneration, although mild edema and inflammatory changes persisted (Fig. 3f). The mean inflammatory scores were significantly reduced in the control and colitis + 1000 mg MG groups compared with the colitis group ($p < 0.01$, Fig. 4f). However, the colitis + sulfasalazine group did not show statistical significant difference when compared with the other groups ($p > 0.05$, Fig. 4f). Mean microscopic scores for histopathological parameters were shown in Fig. 4f.

7.1 Biochemistry

A significant increase in Na^+/K^+-ATPase levels was recorded in the AA group relative to the control group ($p = 0.03$), while a decrease was detected in the 300 mg and 1000 mg MG groups ($p = 0.042$). Na^+/K^+-ATPase levels were significantly reduced in the 300 mg MG and 1000 mg MG groups ($p < 0.001$) compared to the AA group. A notable elevation was observed in the 100 mg MG group ($p < 0.001$) when compared to the sulfasalazine group, (Fig. 4a; Table 2). MMP-3 levels were higher in the AA group than in the control; however, this difference was not statistically significant. In contrast, a significant decline was noted in the 300 mg MG and sulfasalazine groups when compared to the AA group ($p = 0.02$). The 100 mg MG group demonstrated a statistically significant increase relative to the sulfasalazine group ($p = 0.012$) (Fig. 4b; Table 2). Caspase-3 levels were significantly elevated in the AA group compared to the control ($p < 0.001$). Caspase-3 levels in the 100 mg MG, 1000 mg MG ($p = 0.033$), and sulfasalazine groups were significantly reduced compared to the AA group (Fig. 4c; Table 2). A substantial increase in caspase-9 levels was observed in the AA group in comparison to the control group ($p < 0.001$), while a reduction was recorded in the sulfasalazine group ($p < 0.01$). Caspase-9 levels in the 100 mg, 300 mg, 1000 mg MG, and sulfasalazine groups were significantly reduced compared to the AA group ($p < 0.001$). In comparison to the sulfasalazine group, significantly elevated levels of caspase-9 were noted in the 100 mg and 1000 mg

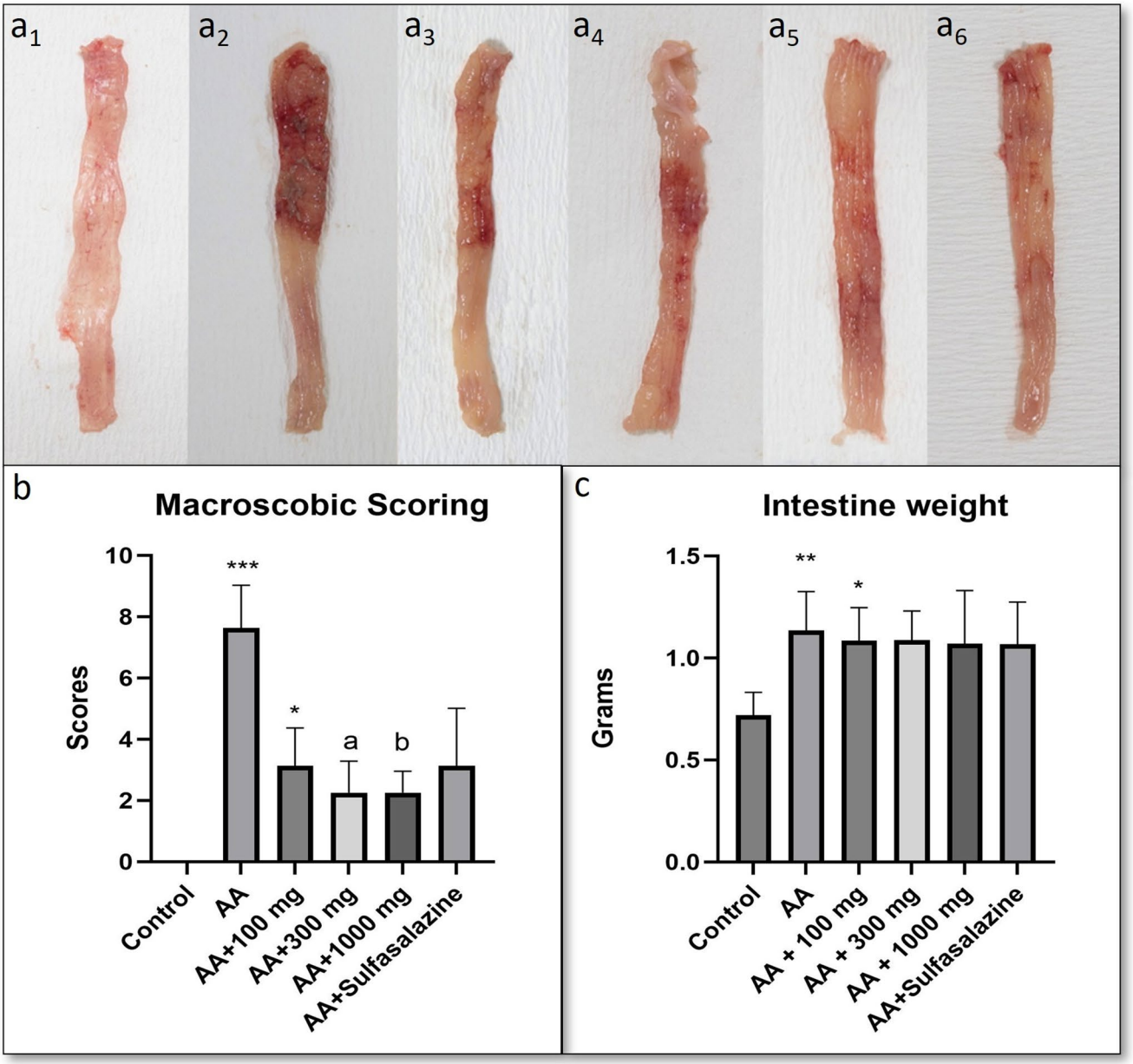


Fig. 2 Representative macroscopic images of colon tissues obtained from each experimental group at the end of the study are shown for scoring (**a1**: control group without colitis induction; **a2**: colitis group induced by acetic acid (AA); **a3**: AA+MG 100 mg/kg; **a4**: AA+MG 300 mg/kg; **a5**: AA+MG 1000 mg/kg; **a6**: AA+sulfasalazine 100 mg/kg. (**b**) Macroscopic damage scores. *$p < 0.05$; ***$p < 0.001$ vs. control group; a, $p < 0.05$; b, $p < 0.01$ vs. AA-induced colitis group. (**c**) Intestine weight. *$p < 0.05$; **$p < 0.01$ vs. control group. All data are presented as mean ± S.E.M. ($n = 8$ per group). Statistical analyses were performed using the Kruskal–Wallis test followed by Dunn's post hoc test with Bonferroni correction

MG groups (Fig. 4d; Table 2). TLR-9 levels were significantly increased in the AA group compared to the control ($p < 0.001$), whereas a significant reduction was observedin the 1000 mg MG and sulfasalazine groups ($p < 0.001$). The levels of TLR-9 in the 100 mg, 300 mg, 1000 mg MG, and sulfasalazine groups were significantly lower than those in the AA group ($p < 0.001$). The 100 mg and 300 mg MG groups showed significantly elevated TLR-9 levels than the sulfasalazine group (Fig. 4e; Table 2).

Analysis of inflammatory markers indicated a significant decrease in TNF-α levels in the 300 mg MG group relative to the AA group ($p = 0.044$) (Fig. 5a; Table 3). A significant increase in IL-17 levels was noted in the AA group compared to the control ($p < 0.001$). IL-17 levels were significantly reduced in the 100 mg, 300 mg, 1000 mg MG, and

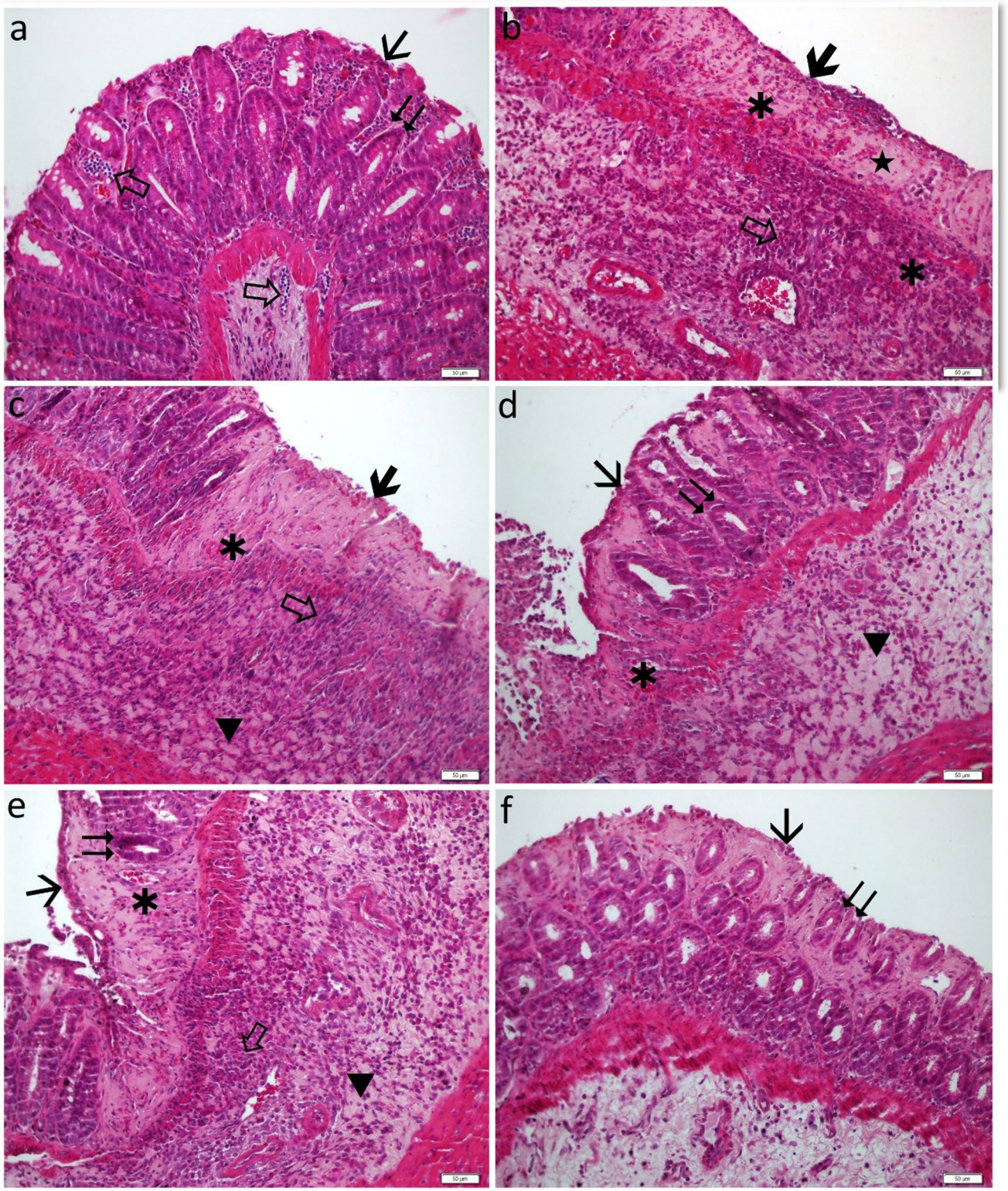
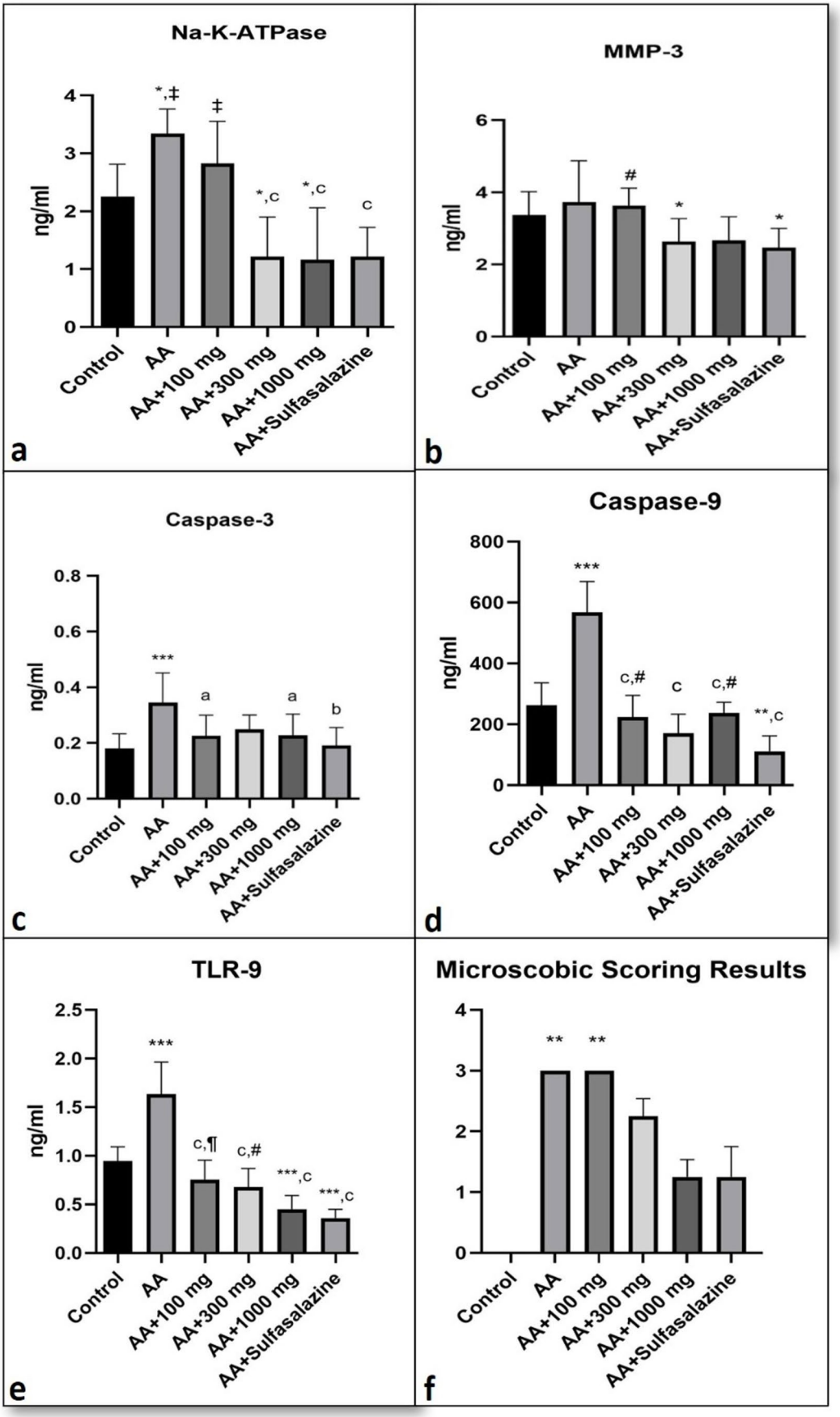


Fig. 3 Representative histological images of colon tissue sections obtained from each experimental groups (**a**: control group without colitis induction, **b**: colitis group induced by acetic acid (AA), **c**: AA+MG 100 mg/kg, **d**: AA+MG 300 mg/kg, **e**: AA+1000 MG mg/kg, **f**: AA+sulfasalazine 100 mg/kg. Surface epithelium (thin arrow), glands (double arrow), lymphocyte infiltration (open arrow), distorted epithelium and glands (thick arrow), necrotic foci (star), hemorrhage (asteriks) and edema (arrowhead) are seen (AA: acetic acid, MG: *Mespilus germanica*, Hematoxylin and eosin. Scale bars: 50 µm)


Na-K-ATPase
ng/ml
*,‡
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*,c
c
Control
AA
AA+100 mg
AA+300 mg
AA+1000 mg
AA+Sulfasalazine
a
MMP-3
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Fig. 4 Tissue damage and apoptosis markers including Na^+/K^+-ATPase (**a**), MMP-3 (**b**), Caspase-3 (**c**), Caspase-9 (**d**), and TLR-9 (**e**) levels among the study groups. Sample size $n=8$ for each group; ANOVA test were conducted using using Tukey's multiple comparison test as a statistical method. Microscobic scoring results of tissue injury across all groups (**f**). Semi-quantitative evaluation was performed using four parameters: mucosal degeneration (surface epithelium, glands, lamina propria), hemorrhage, interstitial edema, and inflammatory cell infiltration. Each parameter was graded on a 4-point scale (0 = none, 1 = mild, 2 = moderate, 3 = severe), and mean scores for each group are presented (AA: Asetic acid; **, $p<0.01$)

sulfasalazine groups compared to the AA group ($p<0.001$) (Fig. 5b; Table 3). IL-1β levels were markedly elevated in the AA group relative to the control ($p<0.01$). A notable increase was observed in the 100 mg MG group. Notable reductions were detected in the 300 mg and 1000 mg MG groups. The 300 mg, 1000 mg MG, and sulfasalazine groups exhibited substantial decreases relative to the AA group ($p<0.001$) (Fig. 5c; Table 3). No significant difference in IL-10 levels was observed in the AA group compared to the control; however, significant decreases were noted in the 300 mg, 1000 mg MG, and sulfasalazine groups ($p=0.014$, $p<0.01$, and $p=0.02$, respectively). Additionally, the 300 mg, 1000 mg MG, and sulfasalazine groups showed significant reduction in IL-10 levels compared to the AA group ($p=0.024$, $p<0.01$, and $p=0.041$, respectively) (Fig. 5d; Table 3).

No significant differences were observed in the levels of MPO and LP across the groups (Fig. 6a, b; Table 4). The levels of MnSOD were significantly higher in the AA group compared to the control ($p<0.01$), and significantly lower in the sulfasalazine group ($p<0.01$). In comparison to the AA group, MnSOD levels were significantly lower in the 100 mg, 300 mg, 1000 mg MG, and sulfasalazine groups ($p<0.01$, $p<0.01$, $p<0.001$, and $p<0.001$, respectively). However, when compared to sulfasalazine group, significant increases were noted in the 100 mg and 300 mg MG groups ($p<0.01$) (Fig. 6c; Table 4). GSH levels were significantly lower in the AA group than in the control group ($p=0.042$) (Fig. 6d; Table 4).

7.2 In Vitro Antioxidant, Anti-inflammatory Activity and Phytochemical Evaluation of the Extract

The extract exhibited low antioxidant activity against the DPPH radical, with an IC_{50} value of 1251.00 µg/mL, in comparison to the standard (Ascorbic acid IC_{50}: 40.23 µg/mL). Similarly, the extract displayed lower antioxidant activity against the $ABTS^+$ radical cation, with an IC_{50} value of 515.3 µg/mL compared to the standard (Trolox IC_{50}: 4.54 µg/mL). Compared to the standard (Indomethacin IC_{50}: 21.42 µg/mL), the extract showed a strong anti-inflammatory effect against the lipoxygenase enzyme, with an IC_{50} value of 17.76 µg/mL. The standard gallic acid calibration curve equation (y = 0.093x + 0.062 [R2: 0.9987]) was used to calculate the total phenolic content of the extract in gallic acid equivalents (GAE). According to this equation, the extract was found to contain 7.35 mg GAE of total phenolic compounds per gram of extract. The total flavonoid content of the extract, expressed as quercetin equivalents (QE), was calculated using the standard quercetin calibration curve equation (y = 0,003x-0,015 [R2:0.9644]). The extract was determined to contain 33.80 mg QE of total flavonoid compounds per gram of extract, based on this equation. The standard oleanolic acid calibration curve equation (y = 0,027x-0,016 [R2:0.9981]) was employed to determine the total triterpene content of the extract, which was expressed as oleanolic acid equivalents (OE). As per this equation, the extract was found to contain 79.96 mg OE of total triterpene compounds per gram of extract (Table 5).

8 Discussion

Ulcerative colitis (UC) is an inflammatory bowel disease that affects the rectum and colon. Mucosal inflammation initiates in the rectum and may extend to the proximal segments of the colon in patients with UC [2]. In our study, UC symptoms were induced by IR administration of AA. Inflammation and tissue damage caused by colitis lead to intestinal edema, resulting in an in bowel weight. In our model, AA-induced UC led to evident macroscopic and microscopic alterations in the colon. These included increased colon weight, a substantial increase in ulcer index, mucosal ulceration, edema, and the infiltration of inflammatory cells, all of which eventually resulted in the loss of mucosal glandular crypts. These findings were consistent with the observations that have been reported in previous studies [7, 45, 46]. However, the lack of a significant reduction in intestinal weight in all MG treatment groups suggested that MG was not effective enough to completely alleviate the inflammation-associated edema. This observation may also indicate that epithelial repair processes were initiated while residual submucosal edema and inflammatory infiltration persisted, which may mask potential reductions in tissue weight during early stages of mucosal recovery. In the AA group, severe edema and inflammation were observed; however, the administration of MG at an oral dose of 300 mg/kg/day resulted in moderate inflammatory cell infiltration and edema in the submucosa, as well as indicators of regeneration in the surface epithelium and glands of the mucosal layer. MG further enhanced mucosal regeneration involving both the surface and glandular epithelium at a higher dose of 1000 mg/kg/day. The coexistence of epithelial regeneration with persistent submucosal inflammation may reflect the

Table 2 Tissue damage and apoptosis markers

	Control	AA	AA+100 mg MG	AA+300 mg MG	AA+1000 mg MG	AA+ Sulfasalazine
Na/K-ATPase (ng/ml)	2.26±0.21	3.43±0.14*	2.82±0.26$^{\&}$	1.22±0.24^{*c}	1.16±0.32^{*c}	1.22±0.19^{c}
MMP-3 (ng/ml)	3.37±0.23	3.73±0.40	3.63±0.17$^{\#}$	2.64±0.22^{a}	2.67±0.23	2.47±0.19^{a}
Caspase-3 (ng/ml)	0.18±0.02	0.34±0.04***	0.22±0.03^{a}	0.25±0.02	0.23±0.03^{a}	0.19±0.02^{b}
Caspase-9 (ng/ml)	262.10±26.26	567.50±35.86***	223.50±25.28$^{c\#}$	170.30±25.68^{c}	237.20±14.29$^{c\#}$	110.80±17.92^{**c}
TLR-9 (ng/ml)	0.94±0.05	1.63±0.12***	0.75±0.07$^{c\$}$	0.67±0.07$^{c\#}$	0.45±0.05^{***c}	0.36±0.03^{***c}

Data are presented as mean±Standard Error of the Mean (S.E.M). Statistical analyses were performed using one-way ANOVA with Tukey's post hoc test for normally distributed data, and Kruskal-Wallis test with Dunn's post hoc test for non-normally distributed data. Compared to control: *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$. Compared to AA: a, $p<0.05$; b, $p<0.01$; c, $p<0.001$. Compared to AA+sulfasalazine: #, $p<0.05$; $, $p<0.01$; &, $p<0.001$

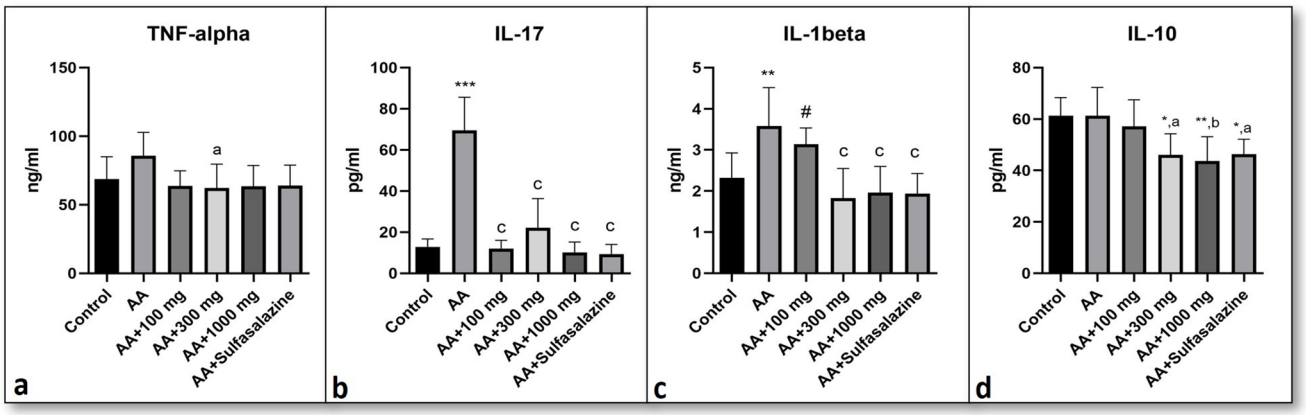

Fig. 5 Comparison of inflammation-related markers TNF-α (**a**), IL-17 (**b**), IL-1β (**c**), and IL-10 (**d**) levels among the study groups. Sample size $n=8$ for each group; ANOVA test were conducted using using Tukey's multiple comparison test as a statistical method

Table 3 Inflammation-related markers

	Control	AA	AA+100 mg MG	AA+300 mg MG	AA+1000 mg MG	AA+ Sulfasalazine
TNF-α (ng/ml)	68.74±6.15	85.81±5.96	63.72±3.94	62.33±6.13^{a}	63.52±5.37	63.92±5.32
IL-17 (pg/ml)	12.89±1.35	69.44±6.12***	12.04±1.40^{c}	22.22±5.35^{c}	10.13±1.81^{c}	9.37±1.64^{c}
IL-1 Beta (ng/ml)	2.32±0.21	3.58±0.33**	3.13±0.15$^{\#}$	1.83±0.25^{c}	1.96±0.22^{c}	1.93±0.17^{c}
IL-10 (pg/ml)	61.28±2.49	61.28±4.16	57.15±3.66	46.00±2.94^{*b}	43.67±3.35**	46.33±2.05^{*a}

Data are presented as mean±Standard Error of the Mean (S.E.M). Statistical analyses were performed using one-way ANOVA with Tukey's post hoc test for normally distributed data, and Kruskal-Wallis test with Dunn's post hoc test for non-normally distributed data. Compared to control: *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$. Compared to AA: a, $p<0.05$; b, $p<0.01$; c, $p<0.001$. Compared to AA+sulfasalazine: #, $p<0.05$; $, $p<0.01$; &, $p<0.001$

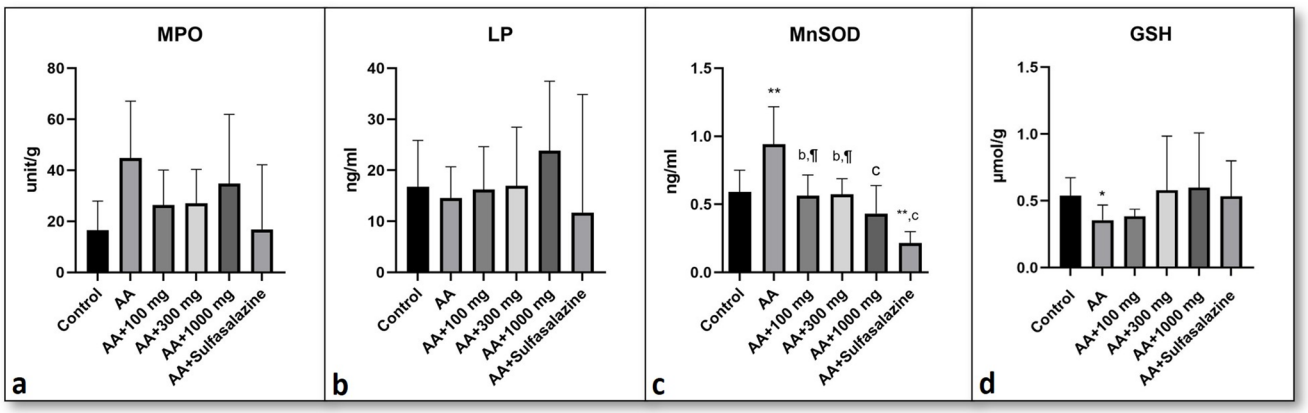

Fig. 6 Comparison of oxidative stress markers MPO (**a**), LP (**b**), MnSOD (**c**), and GSH (**d**) levels among the study groups. Sample size $n=8$ for each group; ANOVA test were conducted using using Tukey's multiple comparison test as a statistical method

Table 4 Oxidative stress markers

	Control	AA	AA+100 mg MG	AA+300 mg MG	AA+1000 mg MG	AA+ Sulfasalazine
MPO (unit/g)	16.52±4.04	44.78±7.89	26.47±4.83	27.05±4.72	34.78±9.58	16.80±8.96
LP	16.74±3.22	14.55±2.17	16.21±2.97	16.94±4.06	23.80±4.83	11.73±8.17
MnSOD (ng/ml)	0.59±0.05	0.94±0.09**	0.56±0.05$^{b\$}$	0.57±0.04$^{b\$}$	0.43±0.07^{c}	0.22±0.03^{**c}
GSH (µmol/g)	0.53±0.05	0.36±0.04*	0.38±0.02	0.58±0.14	0.60±0.14	0.53±0.09

Data are presented as mean±Standard Error of the Mean (S.E.M). Statistical analyses were performed using one-way ANOVA with Tukey's post hoc test for normally distributed data, and Kruskal-Wallis test with Dunn's post hoc test for non-normally distributed data. Compared to control: *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$. Compared to AA: a, $p<0.05$; b, $p<0.01$; c, $p<0.001$. Compared to AA+sulfasalazine: #, $p<0.05$; $, $p<0.01$; &, $p<0.001$

Table 5 In vitro antioxidant, anti-inflammatory activities, and total secondary metabolite content of the 90% ethanol extract of *Mespilus germanica* L. fruit

Experiments	Results	Standards
DPPH radical scavenging activity (IC_{50}, µg /mL)	1251.00±4.24	Ascorbic acid 40.23±2.08
ABTS radical cation scavenging activity (IC_{50}, µg /mL)	515,3±0.28	Trolox 4.54±0.08
Anti-lipoxygenase activity (IC_{50}, µg /mL)	17.76±0.34	Indomethacin 21.42±0.48
Total phenolic content (mg GAE / g extract)	7.35±0.15	
Total flavonoid content (mg KE / g extract)	33.80±0.92	
Total triterpen content (mg OE / g extract)	79.96±2.16	

asynchronous nature of tissue repair in experimental colitis, where epithelial restitution precedes the full resolution of inflammatory edema. However, submucosal edema and inflammation persisted at modest to moderate levels. The onset of recovery was apparent in both macroscopic and microscopic evaluations of the colon. Our results suggested a dose-dependent therapeutic potential of the MG extract, which might be attributed to its potent anti-apoptotic and anti-inflammatory properties.

In villus cell homogenates of rabbits with chronic intestinal inflammation that resembles human IBD, Na^+/K^+-ATPase activity was found to be approximately threefold lower than normal levels [47]. The inhibition of intestinal Na^+/K^+-ATPase activity in patients with active ulcerative colitis may contribute to fluid accumulation in the intestine and, consequently, to the diarrhea observed in this condition [48]. In another study, a significant reduction in Na^+/K^+-ATPase activity was observed in children aged 12–14 years with severe rectal inflammation due to UC, while those with normal rectal mucosa exhibited higher enzyme activity with increasing age [49]. In our study, diarrhea was observed in animals with UC; and Na^+/K^+-ATPase activity was increased. On the other hand, the level of activity in the 300 mg MG treatment group was similar to the level seen with sulfasalazine treatment. Although Na^+/K^+-ATPase activity is typically reduced in active ulcerative colitis, correlating inversely with the severity of mucosal inflammation and diminished Na^+ absorption in both human IBD and experimental colitis models, this relationship is context-dependent and may vary with the stage of injury and tissue remodeling. In classic clinical analyses, colonic Na^+/K^+-ATPase activity decreases as mucosal inflammation worsens, reflecting loss of absorptive capacity and contributing to diarrheal symptoms. However, ion transport regulation is dynamic, and certain inflammatory mediators and intracellular signaling pathways can stimulate Na^+/K^+-ATPase under specific conditions. For example, signaling cascades involving protein kinases such as PKC, NF-κB and downstream prostaglandin synthesis have been shown to modulate Na^+/K^+-ATPase activity in colonic epithelial models, indicating that the inflammatory milieu itself can drive adaptive increases in pump function. In the context of a 72-hour acetic-acid colitis model—when epithelial injury, inflammatory cell infiltration and early reparative responses coexist—an observed rise in Na^+/K^+-ATPase may thus represent a compensatory regulatory response to barrier disruption and altered ionic homeostasis rather than resolution of inflammation per se, integrating both epithelial adaptation and contributions from the inflamed microenvironment [50–52].

MMPs are involved in the initiation of tissue regeneration and the progression of chronic inflammatory conditions. MMP-3 production by intestinal lamina propria mononuclear cells in IBD stimulated by TNF-α, constitutes a major mechanism of tissue damage. Elevated serum levels of MMP-3 have been reported during flare-ups in patients with UC and Crohn's disease [53]. In our study, the increase in MMP-3 levels in UC was not statistically significant; however, a slight elevation was noted, which subsequently decreased to levels comparable to those in the sulfasalazine treatment group following administration of 300 mg MG. The relatively modest increase in MMP-3 observed in our model may be associated with the moderate severity and relatively short duration of the experimental inflammation, since pronounced elevations are typically reported in chronic or severe disease activity. A study conducted in pediatric patients aged 1–18 years demonstrated that serum

MMP-3 levels increased in parallel with disease activity (mild, moderate, and severe), implying the potential utility of MMP-3 as a marker for assessing clinical activity in UC [54]. The fact that MMP-3 levels were not markedly elevated in UC could be associated with the moderate severity of our model. The decrease in MMP-3 levels to those observed in the sulfasalazine treatment group after 300 mg MG administration could be associated with the anti-inflammatory effects of MG.

Apoptosis plays a critical role in the pathophysiology of IBD, and disruption of mucosal barrier integrity during inflammation could result in the apoptosis of the intestinal epithelium [55]. Our study revealed that apoptotic caspase-3 and caspase-9 levels were elevated in the AA group relative to the control, while these levels were declined in the MG treatment groups. In a UC model induced by AA in rats, increased caspase-3 expression has been reported, which was subsequently reduced by various doses of plant extracts, resulting in a decrease in the number of apoptotic epithelial cells [7]. Our findings indicated that elevated levels of caspase-3 and caspase-9 may disrupt epithelial defense, potentially exacerbating mucosal inflammation. However, treatment with MG extract could contribute to preserving mucosal barrier integrity due to its anti-apoptotic features. A study indicated that apoptosis of intestinal epithelial cells increased in the intestinal tissues of patients with active ulcerative colitis relative to controls, with a more pronounced increase in those requiring surgery than in those receiving medication alone. These findings suggested a correlation between epithelial cell apoptosis and disease severity [56]. TLR signalling under certain conditions might be protective. TLR-9 inhibits TLR-4-mediated apoptosis, thereby restricting bacterial entry and maintaining the epithelial barrier [46, 55]. Conversely, a decrease in the number of TUNEL-positive cells was observed in the small intestine injury induced by doxorubicin in wild-type mice using the TLR-9 antagonist [46]. These findings indicate that the role of TLR-9 in intestinal injury is context-dependent and may vary according to the type and stage of inflammatory stimulation. In the present study, TLR-9 levels were decreased in the MG treatment groups together with reduced caspase-3 and caspase-9 levels. This pattern may suggest that attenuation of inflammatory signaling by MG reduces the activation of TLR-mediated pathways associated with epithelial apoptosis. Therefore, the decrease in TLR-9 expression in the treatment groups may reflect suppression of inflammation-driven signaling rather than a direct inhibitory effect on TLR-9 itself. Taken together, these findings support the notion that MG may contribute to the preservation of epithelial integrity through modulation of apoptosis-related pathways.

In our study, no significant difference was observed in MPO and LP levels between the groups. MPO has been reported to have an unanticipated capacity to mitigate cellular damage in in vitro J774A.1 macrophages subjected to H_2O_2 from a glucose/glucose oxidase system [57]. In an in vivo study, the application of thiocyanate, a competitive substrate for MPO, was shown to modulate MPO activity; however, it did not alter the development of colitis in a mouse model induced by dextran sodium sulfate [58]. The similarity in MPO levels between the groups in this study shows that MPO does not contribute to the formation of the colitis model and the treatment process. Our results suggest that the duration of the animal model may affect the degree of increase in oxidative stress in UC. Oxidative stress has been observed to be elevated in UC animal models in various studies [8, 45, 46, 59, 60]. In colitis, neutrophil infiltration and ROS production increase, leading to a rise in superoxide radical formation. This can induce an antioxidant defense system in cells. Therefore, SOD expression increases, attempting to detoxify superoxide radicals. In this context, we believe that the increase in SOD observed in the present study indicates a protective adaptive response developed by the organism against increased oxidative stress [61, 62].

One study reported that in a DSS-induced rat colitis model treated with green tea polyphenols, SOD activity was increased while pro-inflammatory cytokines such as TNF-α and IL-6 were significantly reduced. The researchers suggested that this effect is related to the reduction of oxidative stress and the suppression of inflammatory signalling pathways due to the strong antioxidant properties of polyphenols [63]. Although our study was similar to Oz et al. in terms of cytokine results, SOD levels were found to be increased in the colitis group as a protective adaptive response.

The decreased GSH levels in colitis were restored to control levels with 300 and 1000 mg MG treatment. The extract's low antioxidant activity against in vitro DPPH and ABTS radical cations is consistent with no evidence of an increase in antioxidant capacity with MG. In addition, MG may modulate glutathione metabolism by enhancing GSH synthesis or recycling mechanisms. It should also be noted that chemical assays such as DPPH and ABTS primarily measure direct radical scavenging activity and may not fully represent complex biological antioxidant responses occurring in vivo. We should also clearly state that, according to our findings, the extract obtained from the fruit of *Mespilus germanica* did not exhibit any antioxidant effect.

Various proinflammatory mechanisms have been recognized as significant contributors to the pathogenesis of ulcerative colitis. An imbalance in the production of pro-inflammatory cytokines, including TNF-α, IL-1β, IL-6, IL-12, IL-17, and IL-23, relative to the anti-inflammatory

cytokine IL-10 has been significantly involved in the pathogenesis of IBD [11–15]. Activated macrophages enhance the inflammatory process in colonic inflammation through the increased secretion of TNF-α and IL-1β [63–65]. In our study, the proinflammatory cytokine TNF-α exhibited an increase in ulcerative colitis; however, this change was not statistically significant. In contrast, levels of IL-17 and IL-1β showed significant increases. Administration of 300 mg MG resulted in a significant reduction of TNF-α levels, achieving values comparable to those obtained with the reference drug treatment, consistent with findings from experimental colitis studies utilizing various plant-based compounds [7, 8, 10, 59]. IL-17 is a cytokine that exerts significant proinflammatory effects and is integral to the pathway resulting in mucosal damage through the stimulation of proinflammatory cytokine expression and production in human cells. The study by Fujino et al. identified IL-17 expression in the mucosa within $CD3^+$ T cells and/or $CD68^+$ monocytes/macrophages. It was found that both the mean number of IL-17^+ cells and serum IL-17 levels were significantly elevated in patients with active ulcerative colitis relative to those with inactive disease [66]. Our study revealed that serum IL-17 levels were significantly higher in the colitis group than in the control group. The MG treatment groups exhibited a significant decrease in IL-17 levels relative to the AA group, with levels similar to those found in the sulfasalazine-treated group. The findings demonstrate that MG extract could reduce IL-17 levels. IL-1β levels were significantly decreased after the administration of 300 mg and 1000 mg MG, achieving levels similar to those in the sulfasalazine-treated group [7, 8, 10, 59]. The findings indicate that the inflammation noted in this study is primarily linked to elevated levels of IL-17 and IL-1β. The antiinflammatory effect of MG is dose-dependent at 300 mg and 1000 mg, demonstrated by its capacity to lower proinflammatory cytokines IL-17 and IL-1β to levels similar to those achieved with sulfasalazine, as well as its ability to enhance regeneration alongside histological improvements.

IL-10 levels in the 300 mg and 1000 mg MG treatment groups were similar to those in the sulfasalazine group, while IL-10 levels in the colitis group remained at control levels [8, 10, 59]. Serum IL-10 concentrations in patients with inflammatory bowel disease increased during the remission phase of the disease but subsequently decreased, regardless of the treatment method [66]. Therefore, fluctuations in IL-10 concentrations may reflect dynamic immune regulation during inflammation and resolution rather than a strictly linear relationship with disease severity. In addition, during colitis, macrophages, Treg cells, and Th2 cells increase IL-10 production. If a treatment reduces the inflammatory load, the organism's need for compensatory IL-10 production also decreases. In our study, we believe that the decreased IL-10 levels in the treatment groups indicate a decrease in compensatory production [67]. Therefore, the decreased IL-10 levels in the MG-treated groups reflect the successful termination of the inflammatory process and the restoration of tissue homeostasis and reduced requirement for regulatory IL-10 productions.

The absence of significant changes in IL-10 levels, despite the marked reduction in pro-inflammatory cytokines and histopathological improvement, suggests that the protective effect of MG may not rely on classical anti-inflammatory cytokine upregulation. The process, beginning with a decrease in TLR-9 levels, may have significantly inhibited the activation of pro-inflammatory signaling pathways due to the suppression of nucleic acid-mediated immune stimulation. This results in a decrease in the production of potent pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-17, indicating suppression of the inflammatory response. Thus, since the inflammatory load is reduced, the organism's need for anti-inflammatory IL-10 production is also reduced. The decrease in caspase-9 and caspase-3 levels accompanying this immunosuppression suggests that the mitochondrial intrinsic apoptotic pathway is inhibited and cellular death processes are limited. In addition, the decrease in MMP-3 levels contributes to the preservation of tissue integrity by reducing inflammation-related extracellular matrix destruction, while the decrease in Na^+/K^+-ATPase activity suggests that cellular metabolic activity and related to ion balance are suppressed. When all these findings are considered together, they may explain the mechanism of the anti-inflammatory and anti-apoptotic effects of MG in the partial improvement it provides in AA-induced colitis.

In in vitro studies of the aqueous ethanol extract from MG fruit, it was noted that while the extract demonstrated low antioxidant activity, its anti-inflammatory effect was markedly high. Platzer et al. (2022) reported that single electron transfer (SET)-based analyses, such as ABTS and DPPH assays, measure the electron loss from a free radical ($R\cdot$), resulting in the formation of a radical anion. They further observed that the presence of hydroxyl (–OH) groups in the molecule, particularly in the catechol configuration, enhances radical scavenging activity, and that phenolic compounds exhibit radical quenching effects due to these functional groups [68]. In line with these observations, the low DPPH and ABTS radical scavenging activities, i.e., the limited antioxidant activity observed for the extract in the present study, can be explained by its low total phenolic content. The extract's therapeutic effect on colitis is attributable to its strong anti-inflammatory properties. Furthermore, qualitative analysis of the total secondary compound content in the extract, as indicated by the observed colors (blue for phenolic compounds, orange-red for flavonoid compounds, and purple for triterpenoid compounds)

demonstrated the presence of secondary metabolites in the extract. The quantitative analysis of these secondary metabolites indicated elevated concentrations of flavonoids and triterpenes. Flavonoids and triterpenes have been shown to be effective in the treatment of colitis [69]. The activity of the extract against colitis could be attributed to the flavonoid and triterpene compounds present in the extract.

One of the main limitations of this study is the lack of detailed phytochemical characterization of the extract used. Future studies employing advanced analytical techniques, such as high performance liquid chromatography with diode-array detection (HPLC-DAD) and liquid chromatography–mass spectrometry (LC-MS), are required to identify the main bioactive constituents. Another limitation is that the inflammatory response was evaluated using only a limited number of selected cytokines and histological parameters. This constraint may prevent the findings from fully reflecting the complexity of immune responses and oxidative stress processes in experimental colitis. In this context, future investigations incorporating a broader panel of pro- and anti-inflammatory cytokines, together with advanced histological and molecular analyses, would provide a more comprehensive understanding of the protective mechanisms of natural compounds in experimental colitis.

Future studies should further clarify the mechanisms underlying the protective effects of natural compounds in experimental colitis. In particular, investigations focusing on the modulation of key inflammatory signaling pathways such as NF-κB and Nrf2 may help explain the observed changes in oxidative stress parameters and cytokine profiles. In addition, dose–response and long-term studies are needed to determine the optimal therapeutic range and safety profile of these compounds. It would also be valuable to evaluate their effects in different experimental colitis models, such as DSS or TNBS models, to confirm the reproducibility of the findings. Finally, studies examining gut microbiota alterations and epithelial barrier integrity may provide further insight into the potential therapeutic role of natural products in inflammatory bowel disease.

9 Conclusion

This study is the first to demonstrate that the extract obtained from the fruit of MG shows partial improvement in AA-induced ulcerative colitis. Our findings suggest that, in a 72-hour application, the extract exhibits anti-inflammatory and anti-apoptotic therapeutic effects by suppressing the severity of inflammatory and apoptotic mechanisms.

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Data availability The data sets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare that they have no relevant financial or non-financial interests to disclose.

Ethical Approval This study was approved by the Marmara University Local Ethics Committee for Animal Experiments (Approval No: 28.2023mar). All experimental procedures were conducted in accordance with national and institutional guidelines for the care and use of laboratory animals. The study was designed and reported in compliance with the ARRIVE guidelines.

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