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ORIGINAL ARTICLE

Neuropeptide W Exhibits Preventive and Therapeutic Effects on Acetic Acid-Induced Colitis via Modulation of the Cyclooxygenase Enzyme System

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Abstract

Background The novel peptide neuropeptide W (NPW) was originally shown to function in the control of feeding behavior and energy homeostasis. The aim of this study was to elucidate the putative preventive and therapeutic effects of NPW on colitis-associated oxidative injury and the underlying mechanisms for its action.

Methods Sprague–Dawley rats in the acute colitis groups received NPW (0.5, 1 or 5 µg/kg/day) injections prior to induction of colitis with acetic acid, while the chronic colitis groups were treated after the induction of colitis. In both acute and chronic colitis (CC) groups, treatments were continued for 5 days and the rats were decapitated at the 24th hour of the last injections and colon tissues were collected for assessments.

Results NPW pretreatment given for 5 days before colitis induction, as well as treating rats with NPW during the 5-day course of CC, abolished colonic lipid peroxidation. NPW treatment prevented colitis-induced reduction in blood flow, diminished neutrophil infiltration, and pro-inflammatory cytokine responses. NPW pretreatment only at the higher dose reduced colonic edema and microscopic score and preserved colonic glutathione stores. Elevations in cyclooxygenase (COX) enzyme activity and COX-1 protein level during the acute phase of colitis as well as reduction in COX-2 were all reversed with NPW pretreatment. In contrast, NPW treatment was effective in reducing the elevated COX-2 concentration during the chronic phase.

Conclusions NPW alleviates acetic acid-induced oxidative colonic injury in rats through the upregulation of colonic blood flow as well as the inhibition of COX-2 protein expression and pro-inflammatory cytokine production.

Keywords Neuropeptide W · Ulcerative colitis · Oxidative injury · Inflammation · Cyclooxygenases

Introduction

Inflammatory bowel diseases (IBD), clinically classified as ulcerative colitis (UC) and Crohn's disease, are chronic inflammatory conditions of the gastrointestinal tract that present with remission and exacerbation periods [1, 2]. It is estimated that more than 6–8 million people in the world are diagnosed with IBD and require efficient treatments for improving their physical and psychosocial symptoms [3]. Although the exact pathophysiology of IBD is not clearly elucidated, multifactorial mechanisms including environmental factors, genetic predisposition, immune responses, and gut microbiota are recognized to contribute to its pathogenesis [4]. UC is characterized by an intense inflammatory response of the rectal and sigmoid colon, which includes infiltration of neutrophils and monocytes, overproduction of inflammatory mediators [e.g., tumor necrosis factor (TNF)-α, interleukin β (IL-β), IL-6], and increased release of free radicals along with decreased colonic antioxidant capacity [5–8]. On the other hand, cyclooxygenases (COX) participate in the generation of free radicals, while the overexpressed COX-2 enzyme in the colonic mucosa of IBD patients aggravates the oxidative injury [9, 10]. These

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oxidative changes lead to the disruption of mucous barrier, edema, epithelial injury, ulceration, and bleeding that yield to the morphological changes in the colorectal mucosa. Acetic acid model of experimental colitis is a rapid inducible model of IBD that mimics the pathological and clinical features of human UC that are sustained for a few weeks [11, 12]. Currently, conventional therapy protocols for UC are aimed to prolong the remission periods and to decrease inflammation during the exacerbation periods [7, 13]. However, the outcomes of current standard agents used in the treatment protocol of UC (5-aminosalicylates, corticosteroids, anti-TNF-α, and antibiotics) are limited and can have serious side effects [2, 7, 14]. Thus, alternative treatments with more desirable therapeutic profiles are needed to prevent polypharmacy and improve the quality of life by controlling the severity and progression of disease with fewer hospitalizations [7].

The novel peptide neuropeptide W (NPW), occurring with 23 (NPW23) or 30 (NPW30) amino acid residues, binds to its orphan G-protein-coupled GPR7 (NPBWR1) and GPR8 (NPBWR2) receptors [15, 16]. In parallel with its abundant expression in the central nervous system and the gastrointestinal tract, NPW was originally shown to function in the control of feeding behavior and energy homeostasis [16–19]. NPW increases arterial myogenic tone, heart rate, blood pressure, and plasma levels of epinephrine and norepinephrine in rats, exhibiting its modulatory role in the local and central control of cardiovascular function and sympathetic nervous outflow [20–22]. In addition, central NPW mediates the endocrine response to stress via the regulation of hypothalamus–pituitary–adrenal cortex (HPA) axis [23, 24] and demonstrates an antinociceptive effect [25, 26]. Recently, we have shown that peripherally administered NPW alleviates sepsis-induced oxidative multiorgan injury [27], and protects against gastric and cerebral damage via its anti-inflammatory and antioxidant properties [28, 29]. On the basis of these aforementioned observations, the primary aim of this study was to elucidate the putative beneficial effects of NPW on colitis-associated oxidative injury and the underlying mechanisms for its action. Secondly, it was aimed to compare the preventive and therapeutic effects of NPW when it is given before colitis induction or after the initiation of colitis.

Materials and Methods

Animals

Sprague–Dawley female rats (10–12 weeks old), supplied by the Marmara University Animal Center (DEHAMER), were kept in an air-conditioned room with 12-h light and dark cycles, where the temperature (22 ± 2 °C) and relative humidity (65–70%) were kept constant. The animals were fed a standard pellet with free access to food and water, except for the withdrawal of food for 18 h before colitis induction. The experiments were performed in compliance with the Turkish law on the use of animals in experiments, and the principles and guidelines developed by the New York Academy of Sciences were followed. The experimental procedures were approved by the Marmara University Animal Care and Use Committee (approval: 33.2020.mar).

Experimental Design and Colitis Induction

The rats in the acute colitis (AC) groups ($n = 32$) received intraperitoneal (ip) injections of either saline or NPW (Phoenix Pharmaceuticals, USA) for 5 days prior to induction of colitis and were euthanized at the 24th hour of colitis (Fig. 1). In the CC groups ($n = 32$), injections of either saline or NPW were performed for 5 days after the induction of colitis and the rats were euthanized on the sixth day. In both AC and CC groups, treatments were continued for 5 days with the three doses of NPW (0.5, 1, or 5 µg/kg/day) that were selected on the basis of the previous reports [27, 30], and the rats were decapitated at the 24th hour of the last injections. In both AC and CC groups, a polyethylene catheter (PE-60), with its tip positioned at 8 cm from the anus, was inserted into the colon under ether anesthesia, and acetic acid solution (AA; 1 ml, 5% v/v in saline) was instilled. After a 30-s period of exposure, excess fluid was withdrawn and the colon was then flushed with saline [31]. Control groups received isotonic saline instillation into their colon and received intraperitoneal saline injections for 5 days either prior to or after colonic application. During the follow-up period, body weight and food intake of the rats were recorded daily. At the end of the 6-day protocols, rats were anesthetized for the measurement of colonic blood flow. Immediately after the blood flow measurement, intracardiac blood was obtained and colonic tissues were removed.

Colonic Blood Flow Measurement

Under anesthesia (100 mg/kg ketamine and 10 mg/kg xylazine, intraperitoneal) colonic serosal blood flow was evaluated using a laser Doppler flowmeter (PeriSoft 2.5.5 program, PeriFlux System 5000, Perimed, Sweden) at 30 min before euthanasia (Fig. 1). The probe (Probe 307) was placed on the surface of the colon [32, 33], and a 5-min stabilization time was allowed. Then, the average blood flow was calculated from the following 10 min recording period and expressed in perfusion units (PU).

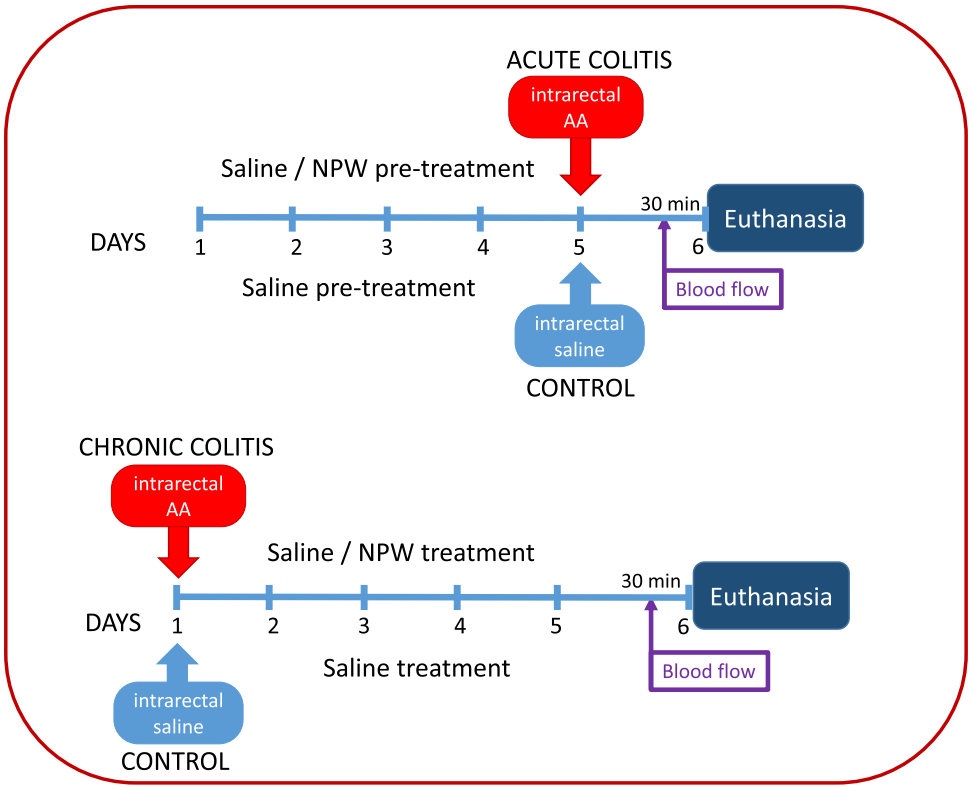

Fig. 1 The design and time course of experimental procedures. *AA* acetic acid. Blood flow was measured using a laser Doppler

Stool Consistency and Macroscopic Damage Scoring in the Colon

Before the rats were anesthetized for blood flow measurement, stool quality was graded using a scale between 0 and 3, where 0 represents normal stool, 1 represents mildly soft stool, 2 represents very soft stool, and 3 represents watery stool [34]. The excised colonic tissues were then evaluated to score the macroscopic damage of the colonic mucosa (no damage, 0; localized hyperemia with no ulcers, 1; single ulceration area without inflammation, 2; single ulceration area with inflammation, 3; <1 cm ulceration areas with inflammation, 4; ≥ 1 cm ulceration areas with inflammation, 5) [35].

Assessment of Colonic Edema

Proximal to the anus, an 8-cm segment of the rectocolonic tissue was dissected and opened longitudinally. The distal 6 cm segment out of the 8-cm dissected colon was weighed to calculate the colon weight/length ratio and then stored for histopathological, biochemical and molecular analyses [35]. To calculate the wet–dry weight ratio, wet weight of the remaining 2-cm proximal colon specimen was measured and reweighed after it was kept at 80 °C for 24 h.

Measurement of Colonic Myeloperoxidase Activity, Malondialdehyde, and Glutathione Levels

Myeloperoxidase (MPO) activity, which is an indicator of tissue neutrophil infiltration, was measured on the basis of H_2O_2-dependent oxidation of *o*-dianisidine.2HCl using a spectrophotometer (PG Instruments Ltd., UK) at 460 nm wavelength, and was stated as units per gram tissue [36]. To evaluate the degree of lipid peroxidation, malondialdehyde (MDA) levels were measured spectrophotometrically at 535 nm wavelength by observing thiobarbituric acid reagent formation and were defined as nmol MDA/gram tissue. Using the modified Ellman procedure, antioxidant glutathione (GSH) levels were measured spectrophotometrically at an absorbance value of 412 nm and the quantity of GSH was given as µmol/g tissue [36].

Measurements of TNF-α, IL-6 Levels, and Cyclooxygenase Activity in the Colon

Colonic levels of the pro-inflammatory cytokines TNF-α (KRC3011) and IL-6 (BMS625) were determined by using the rat ELISA kits according to the manufacturer's (Thermo Fisher Scientific) procedure. COX activity in the colonic samples was performed using the COX activity assay kit (ab204699) according to the manufacturer's

instructions. COX activity was expressed as pmol/min/mg protein in the colonic tissue samples.

Western Blot Analyses for Protein Expressions of Cyclooxygenases in the Colon

Western blot analyses were performed as previously described [37]. Protein concentrations in homogenized colon samples were determined by the Bradford method. Afterwards, 30 µg of protein was resolved in 4% or 12% sodium dodecyl sulfate–polyacrylamide gel electrophoresis and was transferred to PVDF membrane (sc-3718, Santa Cruz Biotechnology, USA), which was blocked with 3% BSA in Tris-buffered saline (TBS), washed twice in TBS plus Tween (TBS containing 0.1% Tween-20), and incubated overnight with the primary antibody (1:500—anti-COX-1: E-AB-61656, anti-COX-2: E-AB-31012, and anti-β-actin: sc-47778). The membrane was incubated with horseradish peroxidase (HRP)-conjugated secondary antibody (1:1000—goat anti-rabbit IgG-HRP: Santa Cruz, sc-2004, and mouse anti-goat IgG-HRP: Santa Cruz, sc-2354) for 2 h. Chemiluminescence reagents (sc-2048, Santa Cruz Biotechnology) using a chemiluminescent imaging system (Syngene, USA) were used to detect the blot. Data were analyzed using the ImageJ OD analysis software. β-Actin was used to normalize the signals.

Microscopic Examination of the Colon

For light microscopic investigations, colonic tissue samples were placed in 10% formaldehyde, dehydrated in ascending alcohol series (70%, 90%, 96%, and 100%), cleared in toluene, and embedded in paraffin. Paraffin sections (5 µm) were stained with hematoxylin and eosin and examined under an Olympus BX51 photomicroscope. Three tissue sections from each of the rats were examined by an experienced histologist (F.E.), who was unaware of the treatments. Assessment of the colonic injury was performed by using the previously described criteria: damage/necrosis (0, none; 1, localized; 2, moderate; 3, severe); submucosal edema (0, none; 1, mild; 2, moderate; 3, severe); inflammatory cell infiltration (0, none; 1, mild; 2, moderate; 3, severe); vasculitis (0, none; 1, mild; 2, moderate; 3, severe); perforation (0, absent; 1, present), with a maximum score of 13 [38].

Statistical Analysis

One-way ANOVA followed by the Bonferroni multiple comparisons test was used to determine the level of statistical significance between experimental groups by using GraphPad Prism 9.3.0 (GraphPad Software, San Diego, CA, USA). All data were presented as mean values with standard error. $p < 0.05$ was considered to be statistically significant.

Results

Induction of AC resulted in soft stool output with increased consistency scores observed at the 24th hour of acetic acid instillation as compared with control group ($p < 0.01$), while the stool quality score in the 6-day CC group was not significantly different from that in the respective control group (Table 1). During the study period, body weight changes and daily food intake were similar between the experimental groups (data not shown). Pretreatment with NPW (0.5, 1, or 5 µg/kg) for 5 days before colitis induction had no significant effect on soft stool output due to AC. Colonic weight/length and wet–dry weight ratios as well as the macroscopic scores were significantly increased in both AC and CC groups when compared with their respective control groups ($p < 0.05$–0.001), demonstrating the presence of colonic mucosal injury and edema. In the CC groups, none of the NPW doses given for 5 days after the colitis induction changed the macroscopically evident mucosal injury or edema. Although the pretreatment with the lower and middle doses of NPW had no significant effect on AC-induced mucosal injury, pretreatment with the 5 µg/kg dose of NPW resulted in a lesser wet–dry weight ratio ($p < 0.05$), which suggest a reduction in acute colonic edema by NPW pretreatment.

Colonic blood flow was significantly depressed in saline-treated AC and CC groups when compared with their respective control groups ($p < 0.05$ and < 0.01; Fig. 2). In the NPW-pretreated groups, colonic blood flow measurements were not significantly reduced and they were not different from those of the control group. On the other hand, blood flow in the colon was significantly elevated with the 5 µg/kg dose of NPW given as a post-colitis treatment ($p < 0.05$), but the increases in colonic blood flow by the lower NPW doses did not reach statistical significance. In accordance with the colitis-induced disruption in blood flow, MDA level, showing lipid peroxidation, and MPO activity, indicative of neutrophil infiltration, were also increased in the colonic tissues of saline-treated AC and CC groups ($p < 0.01$–0.001). When given either before or after the induction of colitis, NPW at all the used doses abolished the increases in MDA levels ($p < 0.01$–0.001; Fig. 2). The 5 µg/kg dose of NPW given as a post-colitis treatment for 5 days significantly decreased colonic MPO activity as compared with that of the respective saline-treated colitis group ($p < 0.05$), suggesting an inhibitory effect of NPW on recruitment of neutrophils during the chronic course of colitis. However, colonic neutrophil infiltration was not changed by either NPW pretreatment or by the lower doses of NPW given as a post-treatment (Fig. 2).

Table 1 Effect of neuropeptide W (NPW) on stool consistency, macroscopic damage score, and colonic edema parameters in acetic acid-induced colitis

	Control	Acute colitis and pretreatment				Control	Chronic colitis and posttreatment			
		Saline	NPW (μg/kg)				Saline	NPW (μg/kg)		
			0.5	1	5			0.5	1	5
Stool consistency	0 ± 0	**1.87 ± 0.47****	**1.50 ± 0.46***	**2.25 ± 0.49****	**2.0 ± 0.32****	0 ± 0	1.13 ± 0.44	0.86 ± 0.45	1.25 ± 0.41	1.29 ± 0.42
Macroscopic score	0 ± 0	**4.62 ± 0.18*****	**4.37 ± 0.26*****	**4.0 ± 0.56*****	**3.62 ± 0.41*****	0 ± 0	**3.25 ± 0.49*****	**2.14 ± 0.50***	**3.5 ± 0.59*****	**2.71 ± 0.47*****
Colonic weight/length ratio (g/cm)	0.10 ± 0.05	**0.21 ± 0.02****	**0.20 ± 0.02****	**0.23 ± 0.03*****	0.16 ± 0.01	0.09 ± 0.00	**0.16 ± 0.01****	**0.15 ± 0.01****	**0.16 ± 0.01*****	**0.16 ± 0.02***
Colonic wet–dry weight ratio	0.13 ± 0.01	**0.38 ± 0.03*****	**0.33 ± 0.03*****	**0.28 ± 0.06***	0.25 ± 0.02^{+}	0.13 ± 0.01	**0.19 ± 0.02***	**0.19 ± 0.01***	**0.19 ± 0.01***	**0.22 ± 0.02****

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, compared with respective saline-treated control group; $^{+}p < 0.05$, compared with respective saline-treated colitis group

Bold values are statistically signifcant values, as it appears.

In parallel with the elevated MPO activity and MDA level, antioxidant GSH content of the colonic tissue was depleted during the first 24 h of colitis induction ($p < 0.01$), and this depletion was also evident on the sixth day of colitis ($p < 0.05$; Fig. 2). NPW pretreatment, only at its higher dose, replenished the colonic GSH content ($p < 0.05$), but NPW given as a post-colitis treatment had no significant effect on the depleted GSH content of the colonic tissue.

In the saline-pretreated AC group, levels of the pro-inflammatory cytokines TNF-α and IL-6 in the colonic tissues were both elevated compared with control group, but only the elevation in IL-6 reached statistical significance ($p < 0.05$; Fig. 3). However, colonic IL-6 levels in NPW-pretreated groups were not different than those of the control group, showing a preventive effect of NPW pretreatment on the production of IL-6. Moreover, in the saline-treated 6-day colitis group, TNF-α and IL-6 levels were significantly elevated ($p < 0.001$ and < 0.05). On the other hand, in the post-colitis NPW-treated groups, colonic IL-6 levels were not different than those of the control group, while TNF-α in the colonic tissues of NPW-treated groups were significantly depressed ($p < 0.5$–0.001), suggesting an anti-inflammatory action of NPW treatment by limiting the cytokine response to CC.

Upon microscopic examination, massive loss of epithelium and glands, severe inflammatory cell infiltration, vasculitis, and submucosal edema were observed in both AC and CC groups, while control groups showed a regular colonic mucosa with normal epithelium, glands, and submucosa (Fig. 4). When NPW was given as a pretreatment or a post-colitis treatment in the AC and CC groups, moderate degrees of mucosal degeneration with localized epithelial and glandular degeneration were evident, demonstrating reductions in inflammatory cell infiltration, vasculitis, and submucosal edema. However, microscopic grading revealed that high injury scores in the saline-treated AC and CC groups were not significantly decreased with the pre- or posttreatment of NPW, except for the higher dose given as a pre- or post-treatment (Fig. 4).

In the saline-treated AC group, colonic COX enzyme activity and colonic COX-1 protein level were significantly elevated as compared with the control group ($p < 0.05$ and < 0.001), while COX-2 protein in the colon was reduced ($p < 0.001$; Fig. 5). To study the involvement of COX enzymes in the protective mechanisms of NPW on colonic injury, the higher and lower doses (0.5 and 5 μg/kg) of NPW were chosen. Pretreatment with both doses of NPW reversed the AC-induced increases in COX enzyme activity and COX-1 level ($p < 0.01$–0.001). Despite that the lower dose of NPW pretreatment in AC further depressed the COX-2 protein level ($p < 0.01$), the higher dose of NPW upregulated the COX-2 level ($p < 0.01$). In contrast to the saline-treated AC group, colonic COX enzyme activity and

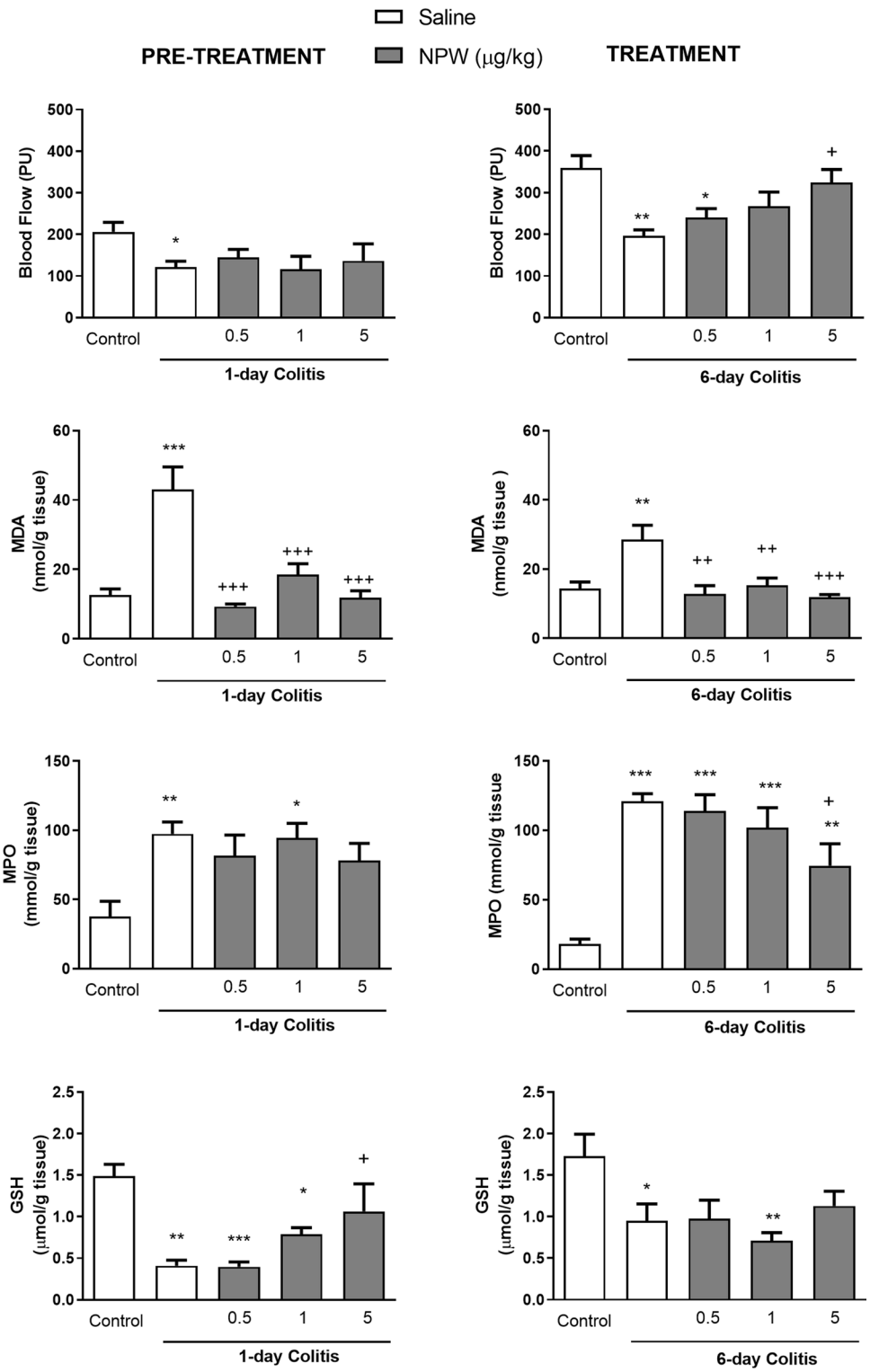

Fig. 2 Colonic blood flow, myeloperoxidase (MPO) activity, malondialdehyde (MDA), and glutathione (GSH) levels in the control, 1-day acute colitis, and 6-day chronic colitis groups that were treated with saline or neuropeptide W (NPW) either as a pretreatment or a post-colitis treatment. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, compared with respective control group; $^{+}p<0.05$, $^{++}p<0.01$, $^{+++}p<0.001$ compared with respective saline-treated colitis group. Each group consists of eight rats

COX-1 protein level in the saline-treated CC group were significantly depressed ($p<0.01$ and <0.001), while the COX-2 level was increased on the sixth day of colitis ($p<0.01$). Treatment with NPW following colitis induction did not alter CC-induced depression in COX enzyme activity, but abolished the elevation in COX-2 level ($p<0.001$). On the other hand, colonic COX-1 protein level was slightly elevated by the lower dose of NPW treatment, but this elevation was not statistically significant when compared with saline-treated colitis group.

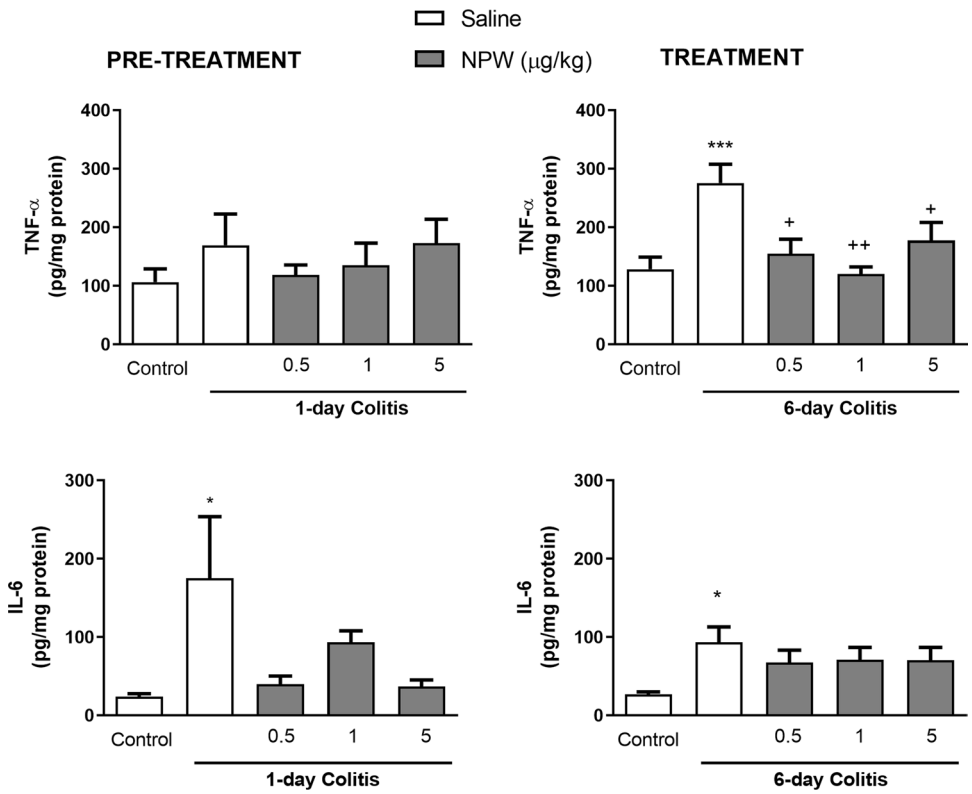

Fig. 3 Colonic levels of TNF-α and IL-6 in the control, 1-day acute colitis, and 6-day chronic colitis groups that were treated with saline or neuropeptide W (NPW) either as a pretreatment or a post-colitis treatment. $^{*}p<0.05$, $^{**}p<0.01$, compared with respective control group; $^{+}p<0.05$, $^{++}p<0.01$, $^{+++}p<0.001$ compared with respective saline-treated colitis group. Each group consists of eight rats

Discussion

The findings of the present study demonstrate that the 5-day NPW pretreatment given before colitis induction, as well as treating rats with NPW during the 5-day course of CC, abolished lipid peroxidation in the colonic tissue. NPW treatment during the CC prevented colitis-induced reduction in blood flow, diminished neutrophil infiltration, and pro-inflammatory cytokine responses. On the other hand, NPW pretreatment only at the higher dose reduced colonic edema and microscopic score and preserved colonic GSH stores. Elevations in COX enzyme activity and COX-1 protein level during the acute phase of colitis as well as reduction in COX-2 were all reversed with NPW pretreatment. In contrast, NPW treatment was effective in reducing the elevated COX-2 concentration during the chronic phase. These results confirm that NPW dose-dependently provides protective and therapeutic effects against acetic acid-induced oxidative colonic injury in rats by upregulating colonic blood flow and by modulating COX enzyme system.

UC is characterized by diffuse inflammation that is limited to the mucosa and submucosa of the colon and rectum [39]. Although the inflammation produced by acetic acid in rodents is not identical to that of human UC, colonic inflammation, epithelial erosions, increased vascular permeability, and neutrophil infiltration are the common pathological features [12, 40–43]. In parallel with human UC, acetic acid-induced colitis is characterized by increased lipid peroxide levels accompanied by elevations of pro-inflammatory cytokines such as TNF-α and IL-6 in colonic tissue [39, 40, 44], making the inhibition of oxidative stress and pro-inflammatory cytokines (e.g., anti-TNF-α) a basic target for the development of therapeutic agents to be used in colitis [45–48]. Accordingly, our data demonstrated that NPW treatment abolished the levels of IL-6 and TNF-α, verifying the anti-inflammatory action of NPW against acetic acid-induced colonic injury. Earlier studies have shown that GSH peroxidase-deficient mice spontaneously develop colitis, while superoxide dismutase-overexpressing mice present with reduced colitis symptoms, showing that oxidative stress is involved in both the onset and exacerbation of colitis and endogenous antioxidants are critical in the pathogenesis of colitis [49, 50]. Similarly, development of colitis in rats causes significant decreases in GSH, as well as superoxide dismutase and catalase activities, verifying that the antioxidant capacity of the colonic tissue is used up during colonic inflammation [51, 52]. Clinical observations have shown that MPO levels in the feces and serum of patients with IBD correlate with the severity of disease and have an important role in aggravating colonic inflammation [53, 54]; thereby, agents that are capable of inhibiting the recruitment of the neutrophils to the inflamed colon could be considered to have a potential therapeutic benefit [6, 55]. The present data revealed that the intraperitoneal administration of NPW,

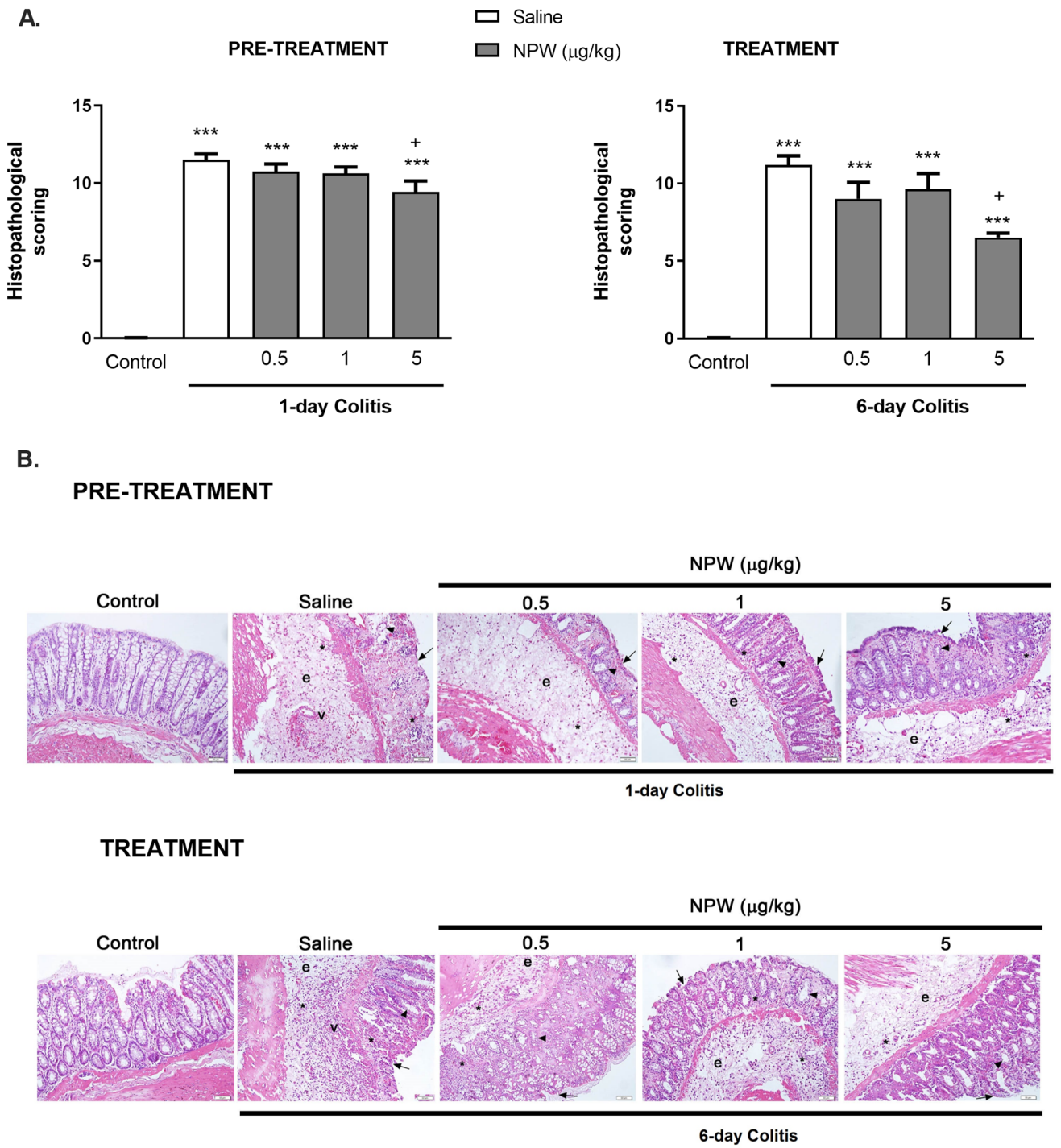

Fig. 4 Microscopic scores (**A**) and representative light micrographs (**B**) in the control, 1-day acute colitis, and 6-day chronic colitis groups that were treated with saline or neuropeptide W (NPW) either as a pretreatment or a post-colitis treatment. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, compared with respective control group; $^{+}p<0.05$, compared with respective saline-treated colitis group. Each group consists of eight rats. Micrographs show massive loss of epithelium (arrow) and glands (arrowhead), severe inflammatory cell infiltration (*), vasculitis (v), and submucosal edema (e) are seen in saline-treated colitis groups. Decreased mucosal degeneration with localized epithelial (arrow) and glandular (arrowhead) degeneration, moderate inflammatory cell infiltration (*) and submucosal edema (e) are seen in the colitis groups that were treated or pretreated with NPW. Hematoxylin and eosin staining. Scale bar, 50 μm. Original magnification, ×200

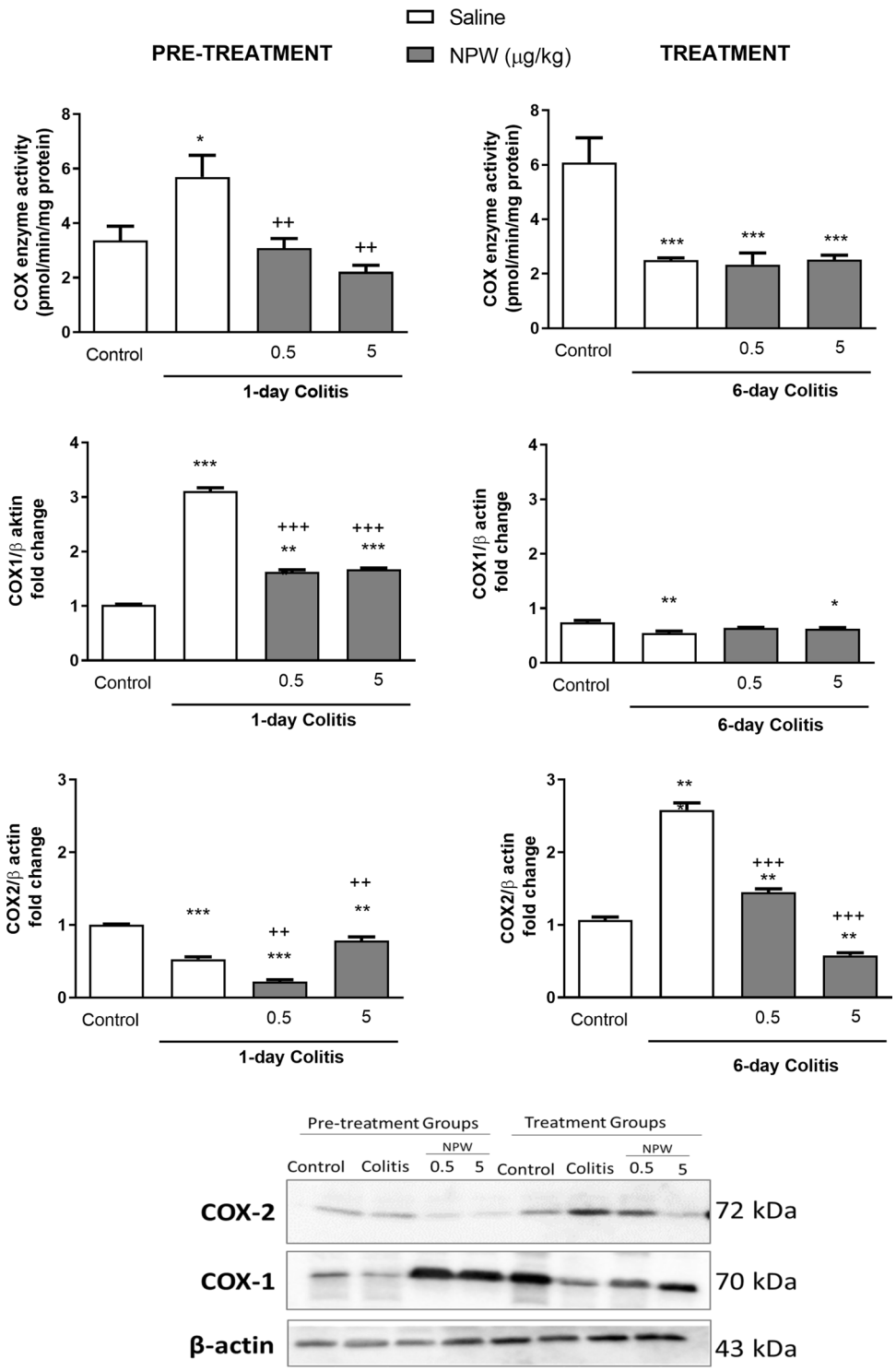

Fig. 5 Colonic COX enzyme activity and COX-1 and COX-2 protein expression levels of colonic tissues in the control, 1-day acute colitis, and 6-day chronic colitis groups that were treated with saline or neuropeptide W (NPW) either as a pretreatment or a post-colitis treatment. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, compared with respective control group; $^{++}p < 0.01$, $^{+++}p < 0.001$ compared with respective saline applied colitis group. Each group consists of eight rats

either before colitis or after colitis induction, abolished lipid peroxidation at all the used doses, showing the inhibitory effect of NPW on oxidative stress of the colonic tissue. However, when given as a pretreatment, only the highest dose of NPW prevented the depletion of GSH. It may be suggested that the lower doses of NPW suppress colonic lipid peroxidation at the expense of GSH stores, while the highest dose of NPW provides an increase in the colonic GSH content. In support of these oxidative parameters, MPO activity was also reduced by the highest dose of NPW in the post-colitis group, suggesting an inhibitory effect of NPW on recruitment of neutrophils during the chronic oxidative course of colitis. Previously, central administration of NPW was shown to alleviate inflammatory pain [26], while its

peripheral administration has exerted anti-inflammatory and antioxidant effects in stress-induced gastric ulcer [30] and hypoxic–ischemic brain injury [29]. Therefore, as observed in other inflammatory models, the protective and therapeutic effects of NPW on colonic injury appear to include its antioxidant and anti-inflammatory actions. Since the NPW signaling system has been demonstrated to be widely distributed in most of the tissues, it was speculated that the blood vessels or the cells of the immune system could be involved in the physiological effects of NPW in all peripheral organs [27], including the mesenteric artery and large intestines. We have previously reported that NPW ameliorates sepsis-induced multiple organ injury by suppressing oxidative stress via the inhibition of NFκB signaling pathway [27]. However, despite the detailed studies in endocrine glands, signaling cascade of NPW in other tissues has not been well clarified yet [56, 57]. Thus, further studies are required to elucidate the signaling mechanisms of NPW in accomplishing its beneficial effects on colonic oxidative injury.

It is well known that disrupted colonic microcirculation is involved in the pathogenesis of many bowel diseases such as ischemic colitis [58]. Although earlier studies in patients with UC have indicated that colonic blood flow rates were highly elevated in severe colitis but decreased in mild colitis [59], decreased colonic blood flow was reported in rats induced with acetic acid colitis [40, 60]. Moreover, agents that could accelerate the healing of colonic damage were shown to reverse acetic acid-evoked reduction in mucosal blood flow [40]. In accordance with these reports, our present data disclosed that colitis-induced reduction in serosal blood flow was improved by NPW in the post treatment colitis groups, suggesting a modulatory role of NPW on gut microcirculation. However, pretreatment with NPW was not capable of preventing acetic acid-evoked acute reduction in blood flow within the first 24 h of colitis, but its beneficial effect could have been observed if a longer follow-up period was allowed to evaluate the blood flow. In parallel with the current findings, we have recently reported that NPW treatment attenuated stress-induced gastric oxidant damage along with an increase in gastric blood flow [28]. It was recently shown that NPW increases myogenic tone by triggering calcium influx via GPR7 receptor, which is co-expressed with L-type calcium channels in the arterial smooth muscle [20]. Since NPW receptors are widely distributed in the peripheral tissues that include blood vessels [61], NPW may have a regulatory role in the maintenance of colonic hemodynamics, which may contribute to the ameliorative effect of NPW treatment in chronic colonic inflammation.

Research has shown that various lipid mediators including prostaglandins are elevated in the colonic mucosa of patients with UC or colitis-induced rodents [62–65], while blockade of prostaglandin synthesis by the nonselective COX inhibitors exaggerates IBD symptoms [66–68]. Although both COX-1 and COX-2 were proven to participate in the inflammatory cascade of IBD, substantial evidence suggests that COX-2 more specifically destructs large intestinal mucosa by upregulating the generation of free radicals and suppressing endogenous antioxidants [63, 69]. Moreover, colonic COX-2 expression in colitis-induced rodents was shown to be increased at the inflammation site, and COX-2 inhibition was proposed to have a promising potential in the treatment of colitis [70–74]. Similarly, surgical resections showed that COX-2 was overexpressed in the apical epithelial cells at the inflamed foci of patients with IBD, while COX-1 expression in IBD was similar to controls [9]. However, endoscopic biopsies of patients with UC at the remission state revealed that COX-2 mRNA was similar to that of the healthy controls and COX-1 expression was downregulated [75]. On the other hand, in the biopsy materials of patients with IBD who were unresponsive to pharmacological treatment, COX-2 expression was reduced with a concomitant elevation in COX-1 [76]. Thus, these reports indicate that conflicting alterations in the COX enzyme system throughout the course of UC may be dependent on the fluctuations in the severity of colonic inflammation, during which the patient experiences remissions or flare-ups. Nevertheless, clinical data suggest that complete mucosal healing consists of a normalized activity of COX-2 enzyme and its downstream signaling pathway [75]. Our current data demonstrated that COX-2 enzyme level was reduced when measured at the 24th hour of colitis induction, but it was then elevated on the sixth day of colitis. On the other hand, NPW treatment during the chronic course of colitis suppressed the COX-2 level in parallel with the abolishment of TNF-α and lipid peroxidation, implicating the therapeutic impact of NPW by inhibiting oxidative injury and normalizing COX-2 enzyme level in the inflamed colon. Furthermore, NPW pretreatment before the occurrence of colitis, which prevented lipid peroxidation and preserved the GSH stores, also reversed the changes in COX enzyme activity. Thus, our data revealed that NPW, either as a pretreatment or a post-colitis treatment, exhibited anti-inflammatory and antioxidant effects by a COX-dependent mechanism.

Since the precise etiology of UC is not fully understood yet, its treatment is mainly symptomatic and requires the development of new choices of therapeutic agents to reduce patient discomfort, to diminish the recurrences, and to reduce the risk of developing colorectal cancer in the long term [7, 77]. Considering the data collected in the present work, it can be concluded that NPW alleviates acetic acid-induced oxidative colonic injury in rats through the upregulation of colonic blood flow as well as the inhibition of COX-2 protein expression and pro-inflammatory cytokine production. Thus, these encouraging results implicate that

further experimental and clinical studies need to be conducted to investigate the possibility of utilizing NPW for the treatment of IBD.

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Declarations

Conflict of interest The authors declare no competing interests.

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