



Anti-inflammatory intestinal activity of *Arctium lappa* L. (Asteraceae) in TNBS colitis model

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ABSTRACT

Ethnopharmacological relevance: In Brazilian traditional medicine, *Arctium lappa* (Asteraceae), has been reported to relieve gastrointestinal symptoms.

Aim of the study: In the present study, we investigated the effects of the lactone sesquiterpene onopordopicrin enriched fraction (ONP fraction) from *Arctium lappa* in an experimental colitis model induced by 2,4,6 trinitrobenzene sulfonic acid and performed experiments to elucidate the underlying action mechanisms involved in that effect.

Materials and methods: ONP fraction (25 and 50 mg/kg/day) was orally administered 48, 24 and 1 h prior to the induction of colitis and 24 h after. The inflammatory response was assessed by gross appearance, myeloperoxidase (MPO) activity, tumor necrosis factor alpha (TNF- α) levels and a histological study of the lesions. We determined cyclooxygenase (COX)-1 and -2 protein expressions by western blotting and immunohistochemistry assays.

Results: TNBS group was characterized by increased colonic wall thickness, edema, diffuse inflammatory cell infiltration, increased MPO activity and TNF- α levels. On the contrary, ONP fraction (25 and 50 mg/kg) treatment significantly reduced the macroscopic inflammation scores ($p < 0.05$ and $p < 0.01$, respectively) and morphological alterations associated with an increase in the mucus secretion. Similarly, the degree of neutrophil infiltration and the cytokine levels were significantly ameliorated. Moreover, COX-2 expression was up regulated in TNBS-treated rats. In contrast, ONP fraction (50 mg/kg) administration reduced COX-2 overexpression.

Conclusions: We have shown that the ONP fraction obtained from *Arctium lappa* exert marked protective effects in acute experimental colitis, confirming and justifying, at least in part, the popular use of this plant to treat gastrointestinal diseases.

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1. Introduction

The inflammatory bowel diseases (IBD) comprise two main types of chronic intestinal disorders: Crohn's disease (CD) and ulcerative colitis (UC) (Singh et al., 2012). The etiology of IBD is still not entirely elucidated, but the characteristic disproportionate inflammatory response in the gut may be developed through various mechanisms at the cellular and intracellular levels (Magro

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and Portela, 2010). As well as some other autoimmune diseases, the overproduction of tumor necrosis factor alpha (TNF- α) is prevalent in the inflamed tissues and plays a central role in inflammation (Lee and Fedorak, 2010; Hai et al., 2011). One major consequence of this signaling in the gut is the increased production of prostaglandin E₂ (PGE₂) via cyclooxygenase -2 (COX-2), the inducible isoform of the enzyme, which has been recognized to be a key factor in diverse inflammatory conditions such as IBD (Wu, 2005).

Arctium lappa L. (Asteraceae), popularly known as “bardana”, is used in folk medicine as diuretic, depurative, digestive stimulant and anti-inflammatory (de Almeida A.B. et al., 2012; de Almeida A.B.A. et al., 2012). Recently, various studies have evaluated the

biological properties of this species, which include antiulcerogenic (de Almeida A.B. et al., 2012; de Almeida A.B.A. et al., 2012), antioxidant (Predes et al., 2011), anti-allergic and anti-inflammatory activities (Soh et al., 2011). In addition, arctigenin a bioactive lignin isolated from *Arctium lappa*, has many biological activities such as inhibition of inducible nitric oxide synthase and interleukin-6 (Zhao et al., 2009), interleukin-2 and interferon gamma (Tsai et al., 2011) and TNF- α production in macrophages (Cho et al., 2004). In this sense, Huang et al. (2010) evaluated the anti-inflammatory activity of *Arctium lappa* L in a dextran sodium sulfate (DSS) model, which resembles UC. However, there is no report studying *Arctium lappa* effects in 2,4,6 trinitrobenzene sulfonic acid (TNBS)-induced colitis model, which mimics CD (te Velde et al., 2006). Therefore, the present study was designed to examine the intestinal anti-inflammatory effects of the lactone sesquiterpene onopordopicrin (ONP)-enriched fraction (hereafter ONP fraction) from *Arctium lappa* in an acute TNBS-induced experimental colitis model which shares some histological and biochemical features to the human disease (Morris et al., 1989). Furthermore, we studied macroscopic and histological parameters and analyzed some inflammatory colonic mediators such as TNF- α production, COX-2 expression, and MPO activity.

2. Materials and methods

2.1. Animals and reagents

Male Wistar rats (170–190 g) obtained from the Laboratory Animal Service of the University of Seville (Seville, Spain) were housed individually in Makrolon cages (45 cm long \times 35 cm high \times 20 cm wide), maintained in air-conditioned animal quarters with a 12 h light/dark cycle, and fed standard rodent chow (Panlab A04, Panlab, Seville, Spain) and water *ad libitum* throughout the experiment. All experiments were in accordance with the recommendations of the European Union regarding animal experimentation (Directive of the European Council 86/609/EC), and followed also a protocol approved by the local animal Ethics Committee and the local Government. ONP fraction was isolated and characterized by GC–MS from *Arctium lappa* according de Almeida A.B. et al. (2012); de Almeida A.B.A. et al. (2012). TNBS and others chemicals were obtained from Sigma Chemical (Madrid, Spain), unless otherwise stated.

2.2. Plant material

Leaves of *Arctium lappa* were collected in Mogi Mirim, SP, Brazil in July 2000 by Dr. Ana Beatriz Albino de Almeida. A voucher herbarium specimen was deposited in the Herbarium of the State University of Campinas (voucher number 131.966).

2.3. Induction of colitis

Colitis was induced according to the procedure described by Morris et al., (1989). Briefly, rats were slightly anaesthetized with ether following a 24 h fast, and then TNBS (Sigma-Aldrich Company Ltd., Spain) dissolved in 50% ethanol was instilled into the colon of the animals (10 mg in a volume of 0.25 mL) using a medical-grade polyurethane catheter for enteral feeding (external diameter 2 mm) inserted 8 cm into the anus. Following the instillation of the hapten, the animals were kept in a head-down position for a 1 min to prevent leakage. Different control groups were created for comparison with TNBS/ethanol instillation: rats in the sham group received an enema of physiological saline instead of the TNBS solution, and ethanol group received 0.25 mL of 50% ethanol. ONP fraction (25 and 50 mg/kg p.o.) was

suspended in 12% Tween[®] 80 solution (10 mL/kg animal) and administered by gavage 48, 24 and 1 h prior to the induction of colitis and 24 h after. The doses of 25 and 50 mg/kg of ONP fraction were chosen based in dose-response study previously performed in our laboratory (de Almeida A.B. et al., 2012). Control groups received vehicle in a comparable volume. The animals were sacrificed, using an overdose of anesthetic, 48 h after induction of colitis. The rats were checked daily for behavior, body weight, and stool consistency.

2.4. Assessment of colonic damage

The severity of colitis was evaluated by an independent observer who was blinded to the treatment. For each animal, the distal 10 cm portion of the colon was removed and cut longitudinally, slightly cleaned in physiological saline to remove fecal residues and weighed. Macroscopic inflammation scores were assigned based on clinical features of the colon (Sánchez-Hidalgo et al., 2005). The presence of adhesions (score 0–2), and/or stool consistency (score 0–1) were evaluated according to the criteria of Bobin-Dubigeon et al. (2001). Pieces of inflamed colon were collected and frozen in liquid nitrogen to measure biochemical parameters.

2.5. Histological analysis

For examination with the light microscope we used tissue samples from the distal colon of each animal fixed in 4% buffered paraformaldehyde, dehydrated in grade ethanol, and embedded in paraffin. Thereafter, sections of tissue were cut at 5 μ m on a rotary microtome (Leica Ultracut), mounted on clean glass slides and dried overnight at 37 °C. Sections were cleared, hydrated, and stained with haematoxylin and eosin, Giemsa, and Alcian blue for histological evaluation of colonic damage, cell infiltration and mucus content respectively, according to standard protocols, and the slides were coded to prevent observer bias during evaluation. All tissue sections were examined in an Olympus BH-2 microscope for characterization of histopathological changes.

Photographs taken from colon samples were digitized using Kodak D290 Zoom camera Eastman Kodak Co., USA and Motic[®] Images 2000 release 1.1 (MicroOptic Industrial Group Co., Ltd.; B1 Series System Microscopes). Analysis of the figures was carried out by Adobe[®] Photoshop[®] Version 5.0 (Adobe Systems) image analysis program.

2.6. Immunohistochemical study

Immunohistochemical analysis was performed using the technique described by Sánchez-Hidalgo et al. (2005). Colonic tissues were fixed in 4% buffered paraformaldehyde, dehydrated through graded concentrations of ethanol, embedded in paraffin, and sectioned. Sections (5 μ m thick) were mounted on slides, cleared, and hydrated. All of them were treated with a buffered blocking solution (3% bovine serum albumin) for 15 min. Then, sections were co-incubated with primary antibodies for COX-1 and COX-2 (goat polyclonal, M-19 and M-20 of Santa Cruz Biotechnologies, CA, USA) at a dilution of 1:400 at room temperature for 1 and 24 h, respectively. Sections were washed with phosphate-buffered saline (PBS) and co-incubated with secondary antibody (anti-sheep IgG, peroxidase conjugated, Sigma, Spain) (1:500 in PBS, v/v), at room temperature for 1 h. Thereafter, sections were washed as before and with Tris–HCl 0.05 M, pH 7.66, and then co-incubated with a 3,3'-diaminobenzidine solution in darkness at room temperature for 10 min. Sections were washed with Tris–HCl, stained with haematoxylin according to standard protocols and observed under an Olympus BH-2 microscope.

2.7. Assessment of leukocyte involvement

MPO activity was assessed as a marker of neutrophil infiltration according to the methods of Grisham et al. (1994). In all animals one sample from the distal colon was obtained. Samples were excised from each animal and rapidly rinsed with ice-cold saline, blotted dry, and frozen at -70°C . The tissue was thawed, weighed and homogenized in 10 vol. 50 mM PBS, pH 7.4. The homogenate was centrifuged at 20,000g, 20 min, 4°C . The pellet was again homogenized in 10 vol. 50 mM PBS, pH 6.0, containing 0.5% hexadecyltrimethylammonium bromide (HETAB) and 10 mM ethylenediamine tetraacetic acid (EDTA). This homogenate was subjected to one cycle of freezing/thawing and a brief period of sonication. A sample of homogenate (0.5 μL) was added to a 0.5 mL reaction volume containing 80 mM PBS, pH 5.4, 0.5% HETAB and 1.6 mM 3,3',5,5'-tetramethylbenzidine. The mixture was incubated at 37°C for 5 min and the reaction started with the addition of 0.3 mM H_2O_2 .

Each tube containing the complete reaction mixture was incubated for exactly 3 min at 37°C . The reaction was terminated by the sequential addition of catalase (20 $\mu\text{g}/\text{mL}$) and 2 mL 0.2 M sodium acetate, pH 3.0. The changes in absorbance at 655 nm were measured with a spectrophotometer. One unit of MPO activity was defined as the amount of enzyme present that produced a change in absorbance of 1.0 U/min at 37°C in the final reaction volume containing the acetate. Results were quantified as U/mg tissue.

2.8. TNF- α levels

The TNF- α levels in distal colon tissues were measured by quantitative enzyme immunoassay kits according to the manufacturer's protocol (Quantikine[®] M, R&D Systems). Colon samples were weighed and homogenized at 4°C , after thawing, in 0.3 mL PBS (pH=7.2), 1% bovine serum albumin (BSA) containing 0.01 mg/mL leupeptin, 0.01 mg/mL pepstatin, 0.01 mg/mL aprotinin, and 1 mM phenylmethylsulfonyl fluoride (PMSF). Then they were centrifuged at 15,300g for 10 min. The concentrations of TNF- α were determined in duplicate and the values were expressed as pg/mg tissue.

2.9. Isolation of cytoplasmic proteins and immunoblotting detection

Frozen colonic tissues were weighed and homogenized in ice-cold hypotonic buffer (1.5 mM MgCl_2 , 10 mM KCl, 0.2 mM phenylmethylsulfonyl fluoride (PMSF), 1.0 mM dithiothreitol (DTT) and 10 mM Hepes, pH 7.9). Homogenates were incubated for 10 min in ice and centrifuged (25,000g, 15 min, 4°C). Cytoplasmic proteins were collected from the supernatants. Protein concentration of the homogenate was determined following Bradford colorimetric method (Bradford, 1976). Aliquots of supernatants containing equal amounts of protein (30–50 μg) were separated on 10% acrylamide gel by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. In the next step, the proteins were electrophoretically transferred onto a nitrocellulose membrane and incubated with specific primary antibodies (Santa Cruz Biotechnology, CA, USA) for COX-1 (M-20) at a dilution of 1:2000 and COX-2 (M-19) at a dilution of 1:400. Each filter was washed three times for 15 min and incubated with the secondary horseradish peroxidase-linked anti-goat (for COX-1 and COX-2) (Santa Cruz Biotechnology, CA, USA). To prove equal loading, the blots were analyzed for β -actin expression using an anti- β -actin antibody (Santa Cruz Biotechnology, CA, USA). Immunodetection was performed using enhanced chemiluminescence light-detecting kit (Amersham, Arlington Heights, IL, USA). Densitometry data were studied following normalization to the control (housekeeping gene). The signals were analyzed and quantified by a Scientific Imaging System (KODAK 1D Image Analysis Software).

2.10. Statistical analysis

All values in the figures and texts are expressed as arithmetic means \pm standard error of the mean (SEM) or standard deviation (SD). The data were evaluated with Graph Pad Prism[®] Version 2.01 software. The statistical significance of differences for each parameter among the groups was evaluated by one-way analysis of variance (ANOVA) followed by Dunnett's test. *P* values of < 0.05 were considered statistically significant.

3. Results

3.1. Protective effect of ONP fraction in acute TNBS-induced colitis in rats

Rats treated with TNBS showed prostration, piloerection and hypomotility. Macroscopic inspection of the cecum, colon and rectum showed evidence of severe colonic mucosal damage, with edema, deep ulcerations and hemorrhage. The animals were severely anorexic, with a marked decrease in average food intake compared to those of the vehicle-treated group variations (Table 1). In addition, a significant increase in the weight/length of the rat colon (as an indicator of inflammation), presence of

Table 1

Quantified parameters after administration of ONP fraction (25 and 50 mg/kg p.o.) in rats with acute colitis induced by TNBS intracolonic instillation (10 mg/animal). Colonic parameters were quantified in the Tween group ($n=14$), which received saline instillation. TNBS group ($n=14$) received TNBS intracolonic in a vehicle of 50% (v/v) ethanol; ethanol group ($n=14$) received 50% (v/v) ethanol intracolonic injection. Data are expressed as means \pm SEM. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$, significantly different from Tween; + $p < 0.05$, ++ $p < 0.01$ and +++ $p < 0.001$, significantly different from TNBS.

Group	Body weight changes (g)	Adhesions (score 0–2)	Diarrhea (score 0–1)	Colon weight/colon length (mg/cm)
Tween	14 \pm 5.38	0 \pm 0	0 \pm 0	98 \pm 7
EtOH	30 \pm 17.55+++	1 \pm 0.27	0 \pm 0+	130 \pm 10+
TNBS	-21 \pm 8.19**	6.36 \pm 0.93**	1.42 \pm 0.78++	280 \pm 90***
TNBS+ONP 25	-13 \pm 4.78	2.67 \pm 0.42++	0.83 \pm 0.75	260 \pm 53**
TNBS+ONP 50	-17 \pm 2.64*	3.67 \pm 0.71+	0.85 \pm 0.69	240 \pm 40**

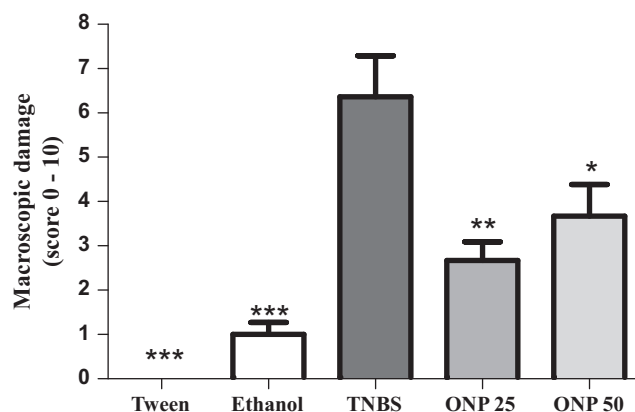


Fig. 1. Effects of acute administration of ONP fraction on the colon damage score. Colonic macroscopic damage resulting from TNBS (10 mg/animal) instilled into rat colon was scored in Section 2. Scores were quantified in the absence of treatment, but with daily administration of the vehicle 12% Tween 80[®] solution (Tween, Ethanol and TNBS groups), and in the presence of ONP fraction (25 and 50 mg/kg/day p.o.). The data are expressed as the means \pm SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. TNBS group.

adhesions to adjacent organs and diarrhea were frequently observed in TNBS-treated rats. On the contrary, ONP fraction treatment (25 or 50 mg/kg) significantly attenuated the extension and severity of the colonic injury, reducing the macroscopic damage score ($p < 0.05$) (Fig. 1), diminished the weight/length of the rat colon and the presence of adhesions (Table 1).

3.2. Histological studies of the colon after ONP fraction treatment on acute experimental TNBS model

The histological features of the colon from sham-treated rats were typical of a normal structure (Figs. 2A and 3A) with normal intestinal cytoarchitecture, epithelial cell layer accompanied by the presence of

goblet cells in straight tubular gland and normal quantity number of cells in lamina propria. On the other hand, the TNBS-treated group showed severe signs of inflammation with transmural necrosis, edema and diffuse inflammatory cells (polymorphonuclear leukocytes, lymphocytes, and eosinophils) infiltration in the mucosa (Fig. 2D). We assessed local ulceration of the colonic mucosa extending through the *muscularis mucosae*, as well as, desquamated areas or loss of the epithelium (Fig. 2D). The architecture of the crypts was distorted and the lamina propria was thickened in peripheral areas of distorted crypts, especially in basal areas (Figs. 2D and 3D).

In order to examine the gel mucus layer, an integral structural-protection of the intestine, the alcian blue staining technique was used. In TNBS-treated group, some areas showed accumulation of

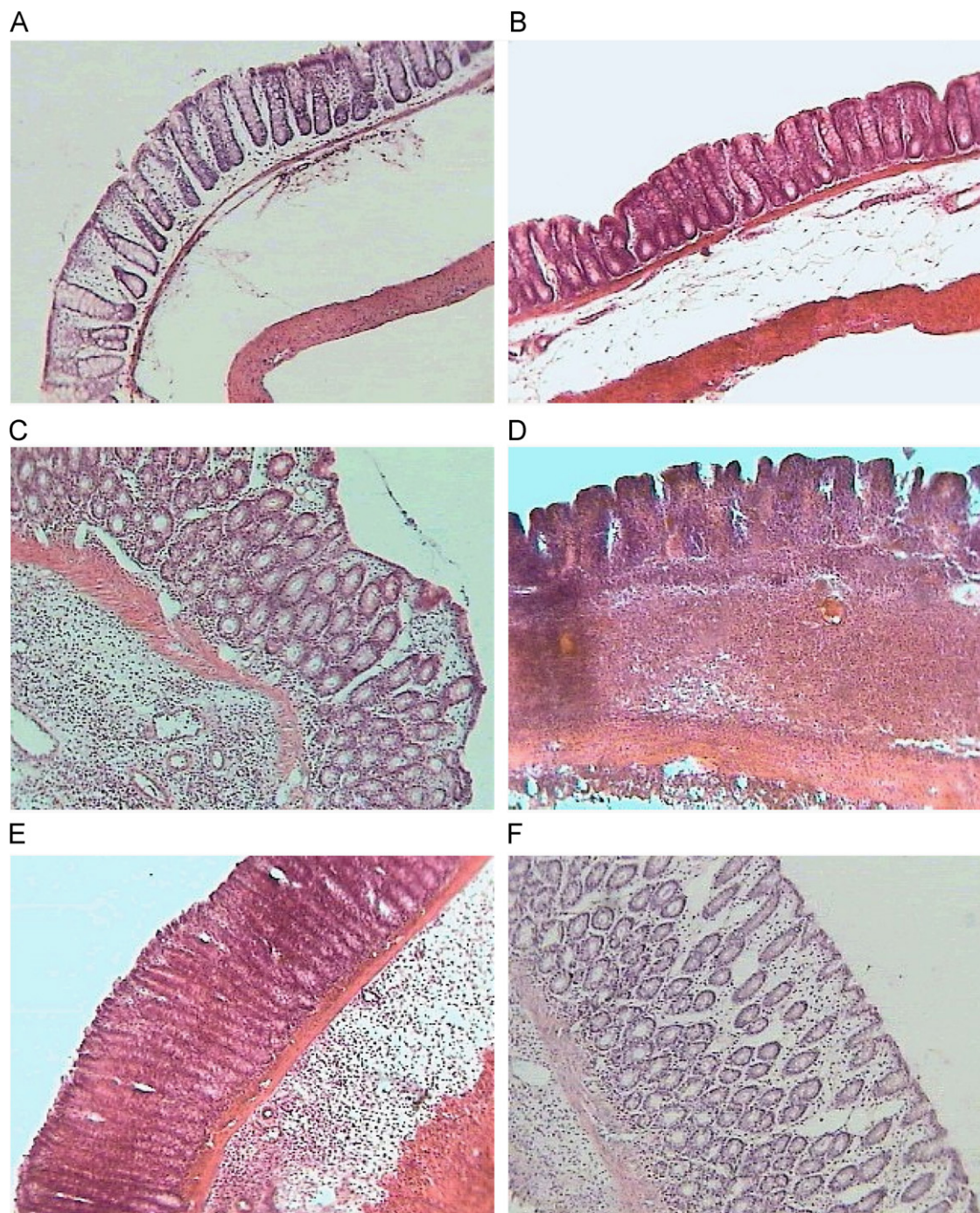


Fig. 2. ONP fraction reduces histological damage. Histological appearance of rat colonic mucosa after haematoxylin and eosin stain: (A) sham group; (B) Tween group; (C) ethanol group; (D) TNBS groups; (E) ONP fraction 25 mg/kg group; and (F) ONP fraction 50 mg/kg group. Original magnification 10x.

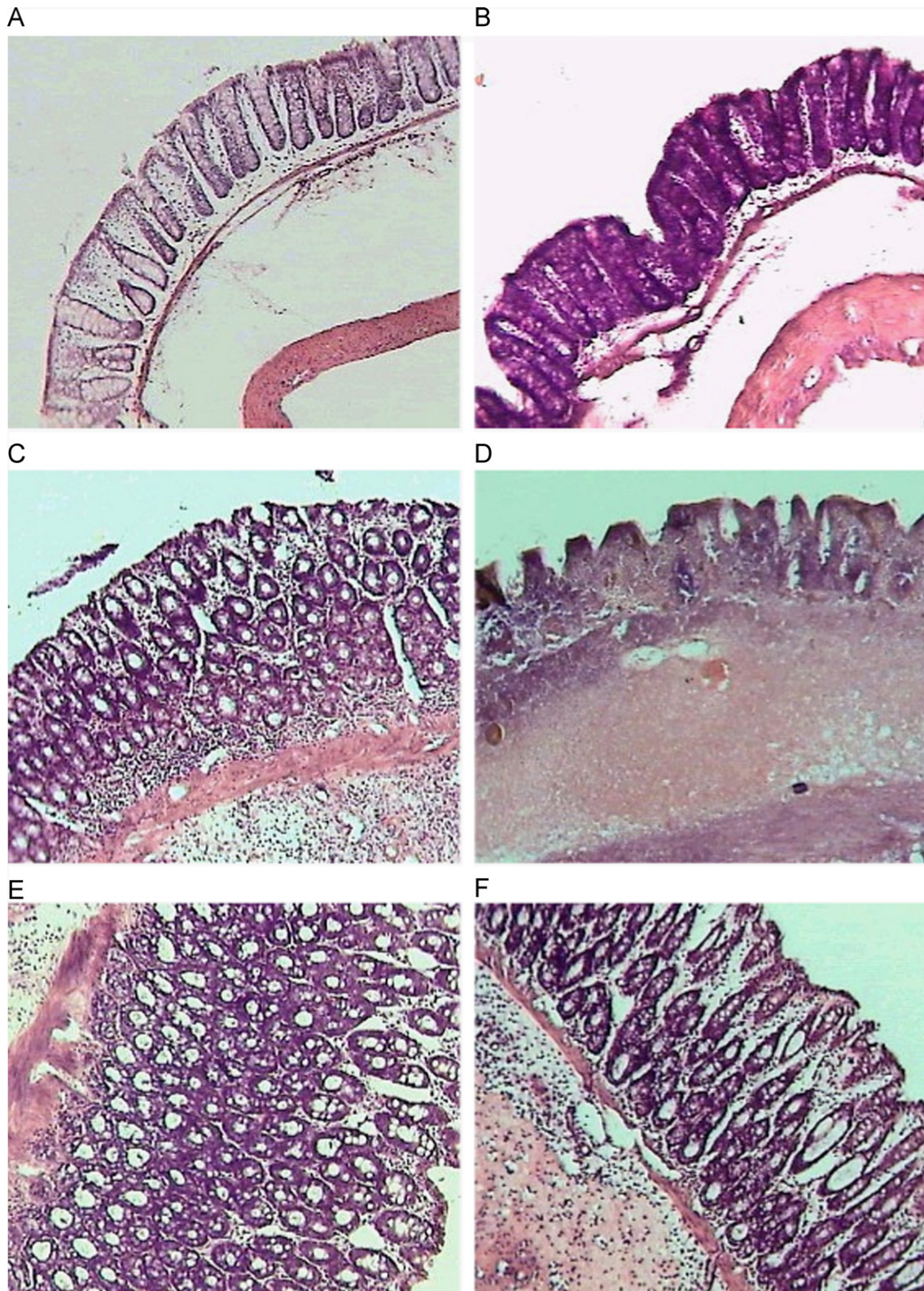


Fig. 3. ONP fraction reduces colonic infiltration in rat colon segments stained with giemsa. Histological appearance of rat colonic mucosa after giemsa stain: (A) sham group; (B) Tween group; (C) ethanol group; (D) TNBS groups; (E) ONP fraction 25 mg/kg group; and (F) ONP fraction 50 mg/kg group. Original magnification 10x.

mucus and cell remnants; however, alcian blue positive cells were less numerous. In fact, in some areas, the mucin layer of the epithelium was missing (Fig. 4D). However, in TNBS-induced rats treated with ONP fraction (25 or 50 mg/kg) some areas of the epithelium remained intact and the mucin layer was clearly visible (Fig. 4E and F).

3.3. Assessment of leukocyte involvement

As shown in Table 2, a marked increase in MPO activity, an infiltration indicator of the colon with polymorphonuclear leukocytes also characterized the colitis induced by TNBS. This result was consistent with the histological findings. Treatment of

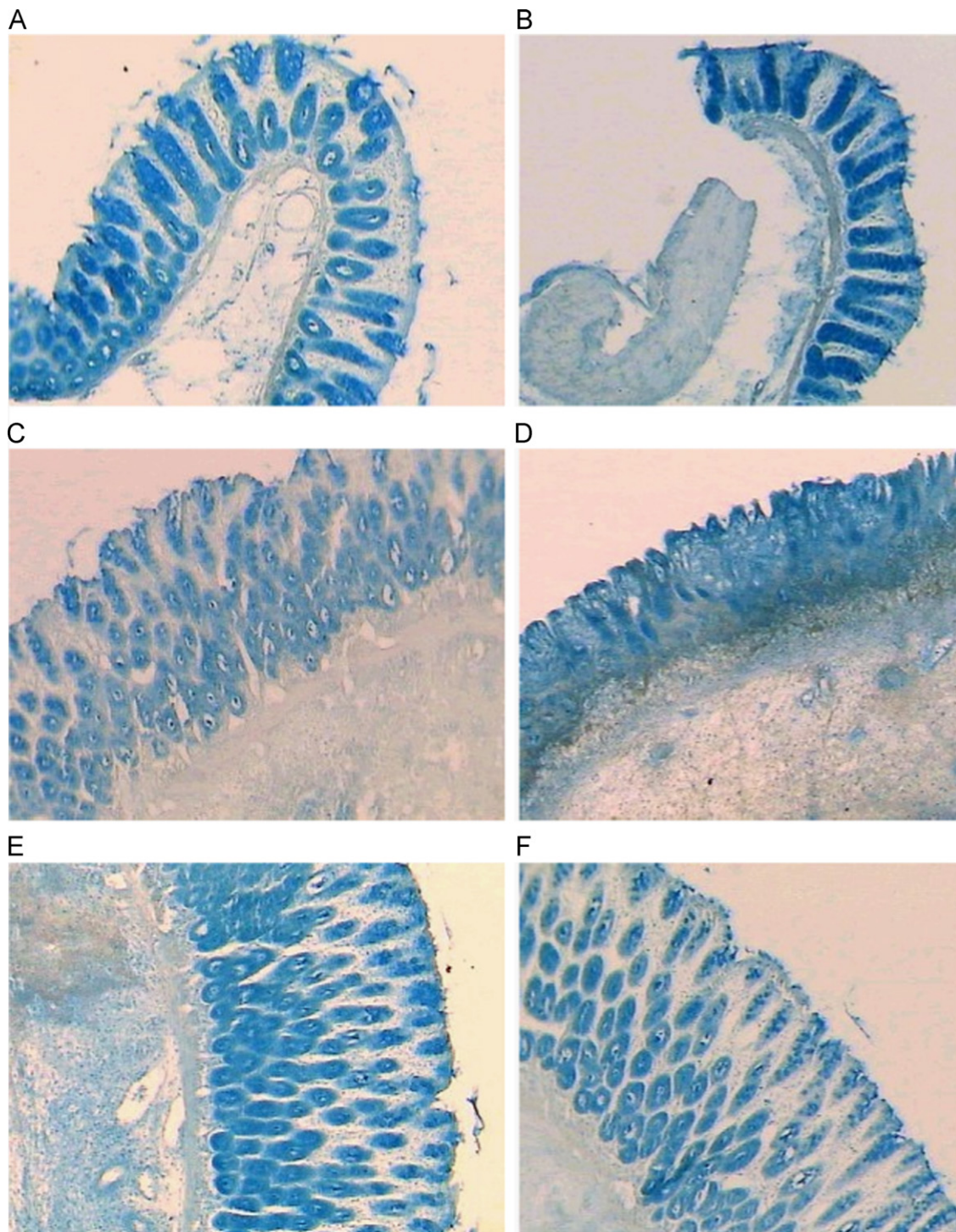


Fig. 4. ONP fraction treatment prevents mucin layer loss. Histological appearance of rat colonic mucosa after alcian blue stain: (A) sham group; (B) Tween group; (C) ethanol group; (D) TNBS groups; (E) ONP fraction 25 mg/kg group; and (F) ONP fraction 50 mg/kg group. Original magnification 10x.

TNBS-treated rats with ONP fraction (25 or 50 mg/kg) significantly ($p < 0.01$ and $p < 0.05$, respectively) reduced the degree of polymorphonuclear neutrophil infiltration.

3.4. TNF- α levels

Colonic injury by TNBS administration was also characterized by an increase in the proinflammatory cytokine TNF- α level (Table 2). In contrast, its levels were significantly lower

($p < 0.01$ and $p < 0.05$) in the groups treated with ONP fraction 25 and 50 mg/kg, respectively.

3.5. Analysis for COX-1 and COX-2 protein expression

The levels of COX-1 and COX-2 expression were measured by Western blotting of cytosolic extracts from colonic mucosa. The levels of COX-1 protein remained unchanged in all groups, indicating that COX-1 protein was constitutively expressed in the colonic tissue and was not significantly changed after TNBS

induction or in presence of ONP fraction (Fig. 5A). On the other hand, COX-2 protein was significantly increased by the hapten ($p < 0.01$), indicating that the inducible isoenzyme expression could be induced at the early stage of colonic lesion caused by TNBS. Nevertheless, oral administration of ONP fraction (50 mg/kg) diminished the up-regulation of COX-2 expression (Fig. 5B).

3.6. Immunohistochemical observations

Specific immunosignals for COX-1 were obtained in the epithelium surface, as well as in the upper half of the crypts from normal colons. Mononuclear cells of the lamina propria and regional lymphatic nodules as well as the cells of the *muscularis mucosae* showed COX-1 specific immunosignals (Fig. 6B) in comparison with negative control (Fig. 6A). In the basal part of the crypts, COX-1 expression was restricted to individual cells, which, according to morphological criteria are endocrine cells, a specialized epithelial cell type of the lower crypt.

COX-2 specific immunolabelling was occasionally observed in colonocytes of the normal surface epithelium of matched control colon as shown in Fig. 7B. Compared with normal colon, significant changes in the cellular distribution of COX-1 and COX-2 were observed in animals treated with TNBS in those colonocytes of the surface and the crypt epithelium, which were only weakly

stained by the COX-1 specific antiserum (Fig. 6D) whereas prominent COX-2 expression was found in cells of the epithelium surface and in the inflammatory infiltrate (Fig. 7D). COX-1 expression reflected no important differences in the cellular localization and the degree of positive staining for COX-1 in colon mucosa from ONP fraction-treated rats after treatment (Fig. 6E and F). At this time, ONP fraction-treated rats showed a lower level of expression of the inducible isoform in apical epithelial cells of inflamed colon (Fig. 7E and F).

4. Discussion

Animal models of intestinal inflammation are indispensable for our understanding of the pathogenesis of CD and UC, the two major forms of IBD in humans (Malik and Mannon, 2012). TNBS model induces inflammation of the rat gut, characterized by several clinical and histopathological features resembling those of human IBD (te Velde et al., 2006).

Actually, the 5-aminosalicylic acid treatment is the first choice for IBD patients, however, these drugs are not entirely effective, they are rather aimed at the remission of the disease and, occasionally, the patients suffer with the worsening of the symptoms (Ford et al., 2011). This aggravation is treated with corticosteroids, but usually followed by heavy side effects and the patients may become either dependent or resistant to the drugs (Ford et al., 2011). In this context, novel treatment possibilities are necessary. It has been widely shown that many plant-derived compounds present significant anti-inflammatory effects, especially when used for the treatment and/or control of chronic inflammatory states such as IBD (Calixto et al., 2004).

The present study confirmed, for the first time, that treatment with ONP fraction was able to reduce the severity and extent of the acute colonic damage induced by TNBS. The decrease in the extent and severity of colitis induced by this hapten was accompanied by a lower incidence of diarrhea and of loss of weight of the animals and decrease in the incidence of adhesions.

Table 2

Myeloperoxidase activity (MPO, U/mg tissue) and tumor necrosis factor alpha levels (TNF- α , pg/mg tissue) after ONP fraction (25 and 50 mg/kg p.o.) in rats with acute colitis produced by TNBS intracolonic instillation (10 mg/kg). Data are expressed as means \pm SEM. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$, significantly different from Tween; + $p < 0.05$, ++ $p < 0.01$ and +++ $p < 0.001$, significantly different from TNBS.

Group	MPO (U/mg tissue)	TNF- α (pg/mg protein)
Tween	0.82 \pm 0.18	0.07 \pm 0.02
TNBS	1.74 \pm 0.03***	0.69 \pm 0.14***
TNBS+ONP 25 mg/kg	0.97 \pm 0.09++	0.12 \pm 0.02++
TNBS+ONP 50 mg/kg	1.18 \pm 0.08+	0.26 \pm 0.04+

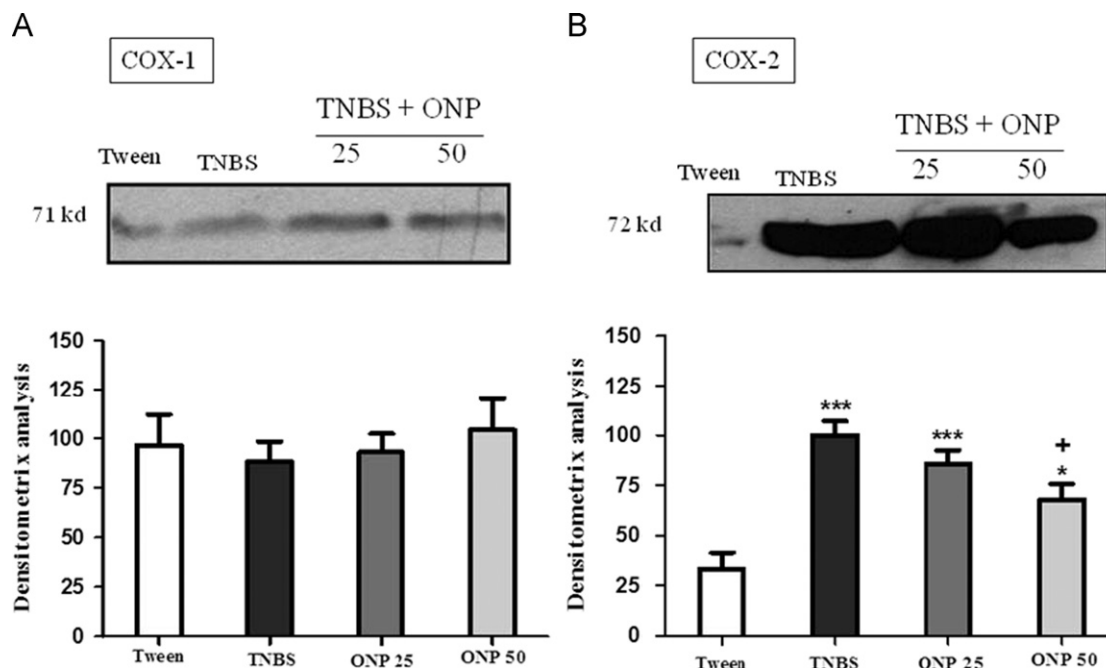


Fig. 5. Analysis of COX-1 and COX-2 protein expressions by western blotting. (A) COX-1 protein remained unchanged in all groups (Tween, TNBS and TNBS+ONP) fraction (25 and 50 mg/kg p.o.), (B) COX-2 was down regulation in ONP fraction-treated groups in comparison with TNBS control group. Densitometry data were studied following normalization to the control (β -actin house-keeping gene). The results are representative of three experiments performed on different samples and data are expressed as mean \pm SEM *** $p < 0.001$ * $p < 0.05$ significantly different from Tween group. + $p < 0.05$ significantly different from TNBS group.

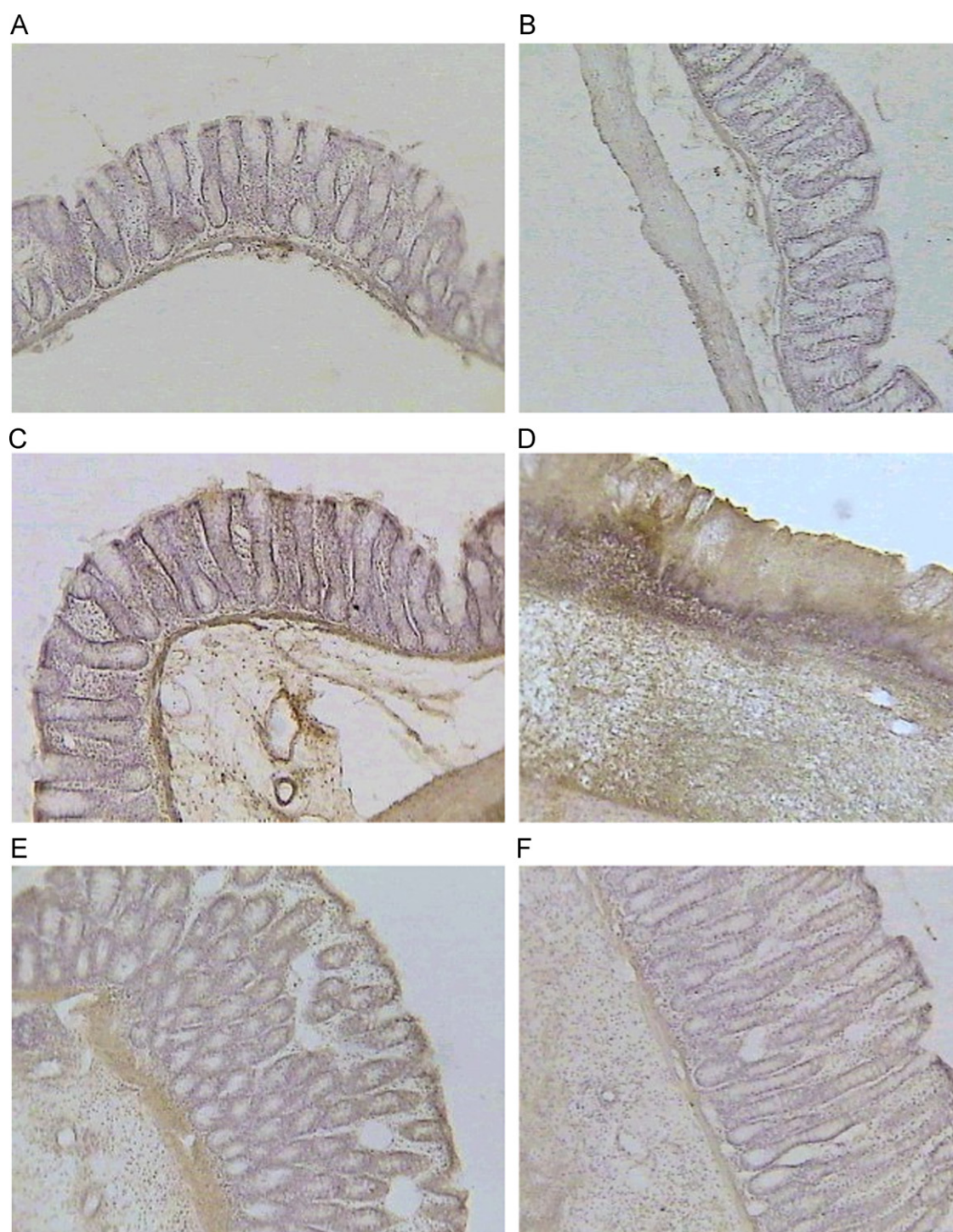


Fig. 6. Immunohistochemical localization of COX-1 isoenzyme in sections of colon. (A) Sham group; (B) Tween group; (C) ethanol group; (D) TNBS groups; (E) ONP fraction 25 mg/kg group; and (F) ONP fraction 50 mg/kg group. Original magnifications 10x.

The presence of adhesions between the colon and adjacent organs, which results from transmural inflammation, is a common feature of TNBS-induced colitis (Morris et al., 1989). The reduction in the incidence of adhesions suggests a beneficial effect of ONP fraction on the extent of the inflammatory process in this experimental model.

Our results also revealed that ONP fraction increased the amount of mucus stained by alcian blue (acid glycoproteins such as sialomucins) in colon mucosa. The mucus layer coating the gastrointestinal tract is the front line of innate host defense, largely because of the secretory products of intestinal goblet cells

(Kim and Ho, 2010). Accumulating evidence indicates that complex interactions are involved in the pathogenesis of IBD including defective mucosal barrier function resulting from abnormal synthesis and/or processing of mucins (Kim and Ho, 2010). Goblet cells are reduced in number and size in UC. Recent studies in murine models of colitis highlighted the importance of mucin at maintaining the integrity of protective mucus barriers whose breakdown can result in colitis (Kim and Ho, 2010). Our findings demonstrate that ONP fraction increased the amount of alcian blue positive cells (acid glycoproteins such as sialomucins) in the colon mucosa. The increase in the intestinal mucus layer may be

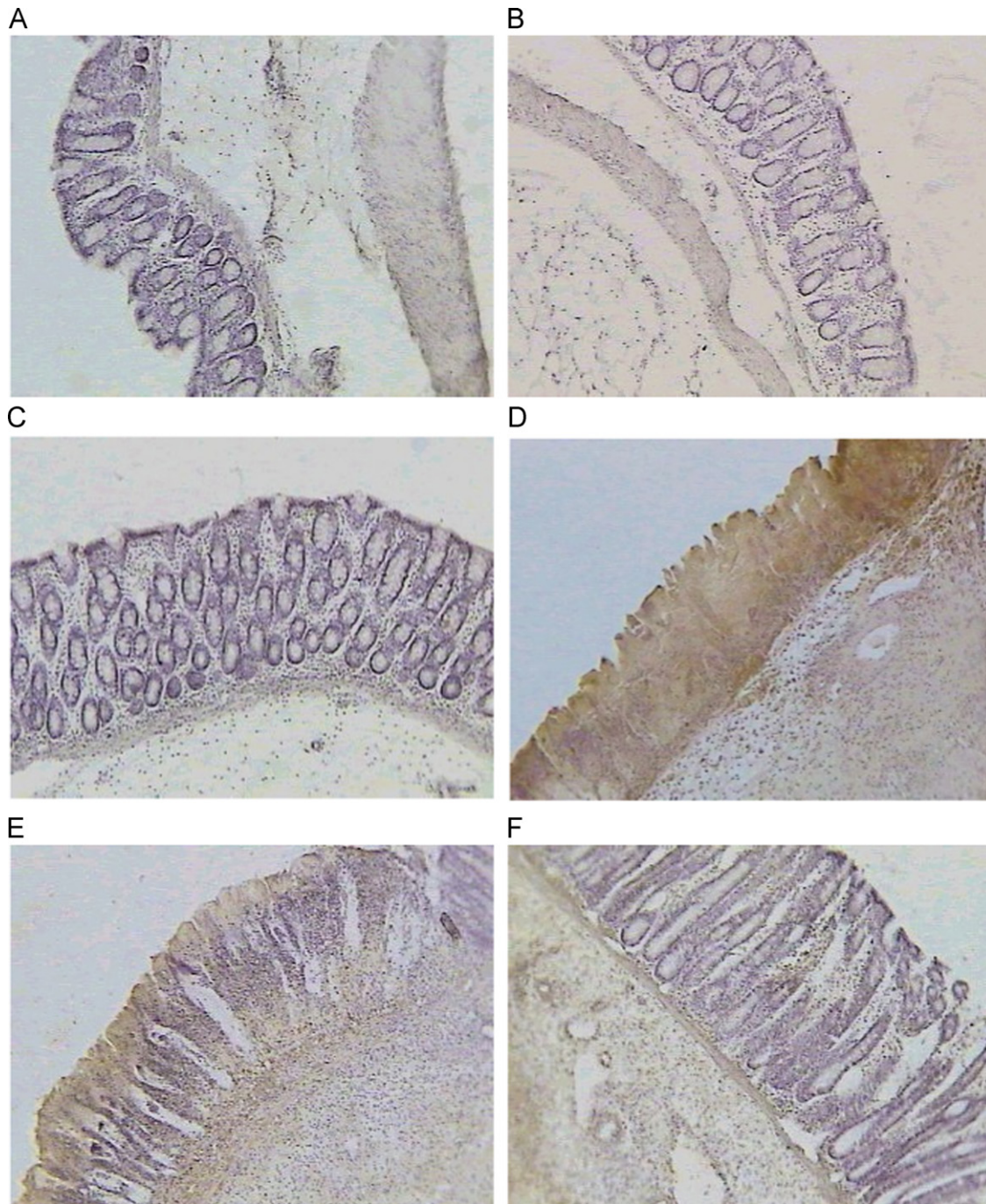


Fig. 7. Immunohistochemical localization of COX-2 isoenzyme in sections of colon. (A) Sham group; (B) Tween group; (C) ethanol group; (D) TNBS groups; (E) ONP fraction 25 mg/kg group; and (F) ONP fraction 50 mg/kg group. Original magnifications 10x.

helpful in the prevention or therapy of intestinal diseases (Kim and Ho, 2010).

Chronic inflammation of the large intestine predominantly comprises lymphocytes and plasma cells exacerbation, neutrophils migrate and degranulate substances like MPO (Masoodi et al., 2011). MPO is an enzyme found in primary granules of polymorphonuclear neutrophils and used as an index for the severity of digestive inflammation (Masoodi et al., 2011). In fact, it is well-known that this enzyme is increased in TNBS and DSS-induced colitis (Lima de Albuquerque et al., 2010; Dutra et al., 2011; De-Faria et al., 2012). On the contrary, ONP fraction treatment led to reduced MPO activity in inflamed colon, pointing out an inhibitory effect on granulocyte infiltration.

The pathogenesis of IBD is originated from a combination of inputs: a genetic predisposition, an environmental trigger, and an abnormal immune response (Kavanaugh et al., 2011). Dysregulation of either immunity or intestinal barrier function allows the initiation of IBD resulting in chronic active inflammation with the production of proinflammatory cytokines such as TNF- α (Lee and Fedorak, 2010; Hai et al., 2011; Kavanaugh et al., 2011). TNF- α is a pleiotropic cytokine that elicits a wide spectrum of physiologic and pathogenic events such as proliferation, differentiation, cell death, modulation of gene transcription, and inflammation (Qidwai and Khan, 2011). Elevated levels of TNF- α have been observed in the serum and feces of CD patients (Yin et al., 2011) and in the experimental colitis (da Silva et al., 2010; De-Faria et al., 2012).

In our experiment, the levels of TNF- α were significantly increased in the colon at 48 h after TNBS instillation. In contrast, the ONP fraction-treated animals showed a significant decline in the TNF- α levels, corroborating with that demonstrated by Huang et al. (2010). However, these authors assayed four-fold higher dose of *Arctium lappa* in comparison with ours. Despite many inhibiting agents have been tested and developed for the IBD treatment, the development of TNF- α inhibitors markedly revolutionized the treatment of IBD. These drugs have proven to effectively induce and maintain remission of the IBD (Wasan and Kane, 2011). Recent studies have demonstrated that other sesquiterpenes are capable of modulating the production of TNF- α (Fonseca et al., 2010; Ferrari et al., 2012; Zhao et al., 2012).

In addition, TNF- α mediates important inflammatory signaling pathways, including the COX-2 expression which plays a key role in various inflammatory pathogenesis of the digestive tract, including colitis (Agoff et al., 2000). COX is rate-limiting in the synthesis of prostaglandins. COX-2 is the inducible isoform of cyclooxygenase, the rate-limiting enzyme that catalyzes the initial step of arachidonic acid metabolic transformation into prostanoids. Studies have shown that inducible enzyme COX-2 is predominantly expressed at sites of inflammation. Increased amounts of this protein have been found in experimental colitis (Lima de Albuquerque et al., 2010; da Silva et al., 2010; De-Faria et al., 2012). Conversely, COX-1 represents the constitutive isoform of COX (Rizzo, 2011; De-Faria et al., 2012). ONP fraction reduced the COX-2 overexpression when compared with TNBS group. Thus, we suggest that the anti-inflammatory activity presented by ONP fraction could be mediated, in part, through COX-2 down-regulation. Zwolinska-Wcislo et al. (2011) showed that the treatment with COX-2 inhibitor decreased the area of colonic lesions, MPO activity and expression of proinflammatory markers such as TNF- α .

5. Conclusion

In summary, our data reveal, for the first time, that pre-treatment with ONP fraction is able to reduce intestinal inflammation in an acute TNBS-induced experimental model of colitis in rats. This anti-inflammatory effect seems to be related to a decrease in the neutrophil function, TNF- α production and COX-2 down-regulation in the intestinal mucosa.

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