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Proteins from *Plumeria pudica* latex exhibit protective effect in acetic acid induced colitis in mice by inhibition of pro-inflammatory mechanisms and oxidative stress

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ABSTRACT

Latex proteins from *P. pudica* (LPPp) have anti-inflammatory activity. In the present study, LPPp was evaluated to protect animals against inflammatory ulcerative colitis (UC). UC was induced by intracolonic instillation of a 6% acetic acid solution and the animals received LPPp (10, 20 or 40 mg/kg) by intraperitoneal route 1 h before and 17 h after acetic acid injection. Eighteen hours after instillation of acetic acid, the mice were euthanized and the colons were excised to determine the wet weight, macroscopic and microscopic lesion scores, myeloperoxidase (MPO) activity, IL1-β levels, glutathione (GSH) and malondialdehyde (MDA) concentration and superoxide dismutase (SOD) activity. The results revealed that LPPp treatment (40 mg/kg) had a protective effect on acetic acid-induced colitis by reducing the wet weight, macroscopic and microscopic scores of intestinal lesions and colonic MPO activity. Additionally, LPPp inhibited tissue oxidative stress, since decreases in GSH consumption, MDA concentration and SOD activity were observed. The treatment with LPPp reduced the levels of cytokine IL-1β, contributing to the reduction of colon inflammation. Biochemical investigation showed that LPPp comprises a mixture of proteins containing proteinases, chitinases and proteinase inhibitors. These data suggest that LPPp has a protective effect against intestinal damage through mechanisms that involve the inhibition of inflammatory cell infiltration, cytokine release and oxidative stress.

1. Introduction

Ulcerative colitis (UC) is an inflammatory bowel disease that involves immune, genetic and environmental factors, related to the initiation and development of colitis [1,2]. During UC there is intense migration of cells to the area of the intestine with chronic inflammation, resulting in overproduction of a variety of pro-inflammatory mediators, including release of pro-inflammatory cytokines, reactive oxygen species and nitrogen metabolites [3–5].

Currently, there is no effective therapy to cure the disease. The palliative treatment involves drugs that have antioxidant activity or the ability to inhibit production of inflammatory mediators, promoting reduction of the abnormal inflammation in the colon [6]. Most people with UC are treated with steroids, immunomodulators and antibiotics to reduce inflammation and relieve symptoms [7]. However, the efficacy and safety of these drugs have recently been questioned because of many side effects [8]. Therefore, it is necessary to find new molecules with significant effect on UC to establish a safe alternative for the treatment of this disease.

Plumeria pudica (Jacq., 1760) is a plant that produces latex

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containing molecules with therapeutic potential. Proteins recovered from this latex (LPPp) inhibited mice paw edema induced by histamine, serotonin, bradykinin or prostaglandin E_2 (PGE_2) and reduced the leukocyte migration, IL-1β, and TNF-α concentration in peritoneal fluid of mice submitted to carrageenan-induced peritonitis [9]. Additionally, LPPp has antioxidant properties by preventing glutathione (GSH) and malondialdehyde (MDA) alterations in inflammatory diarrhea induced by castor oil and PGE_2 [10]. Biochemical evaluation revealed that LPPp comprises mainly proteinases, chitinases and proteinase inhibitors [9,10]. These classes of plant proteins are described in the literature as having anti-inflammatory properties [11–13] and seem to be associated with the beneficial effects of LPPp.

The main goal of this study was to evaluate the protective effect of the LPPp fraction obtained from *P. pudica* latex on inflammatory UC using the experimental model of colitis induced by acetic acid.

2. Material and methods

2.1. Latex collection and processing

The latex was collected in the city of Parnaíba, Piauí, Brazil (2°54′28.8″S; 41°46′35.2″W). The plant material was identified and a voucher specimen (N.2432) was deposited in the Delta do Parnaíba Herbarium of Piauí Federal University. Latex was collected in tubes containing distilled water (1:1; v/v). In the lab, the material was centrifuged at 3600 ×*g* for 15 min at 25 °C. The supernatant was dialyzed against distilled water for 48 h using membranes with 8000 Da cut-off. Then, the material was centrifuged again using the same conditions and the resulting supernatant was lyophilized [9,10]. The material obtained was called latex protein from *Plumeria pudica* (LPPp).

2.2. Biochemical characterization of LPPp

To obtain data about its main constituents, LPPp was submitted to partial biochemical characterization. The protein profile of LPPp was examined through 12.5% polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS) according to [14]. Colorimetric assays were performed to investigate the presence of proteolytic activity and protease inhibitors in LPPp using BANA and BApNA as substrates [15]. Chitinolytic activity was evaluated using chitin-azure as substrate [16]. Results were presented as UA (unit of activity) per μg of protein or percentage (%) of UA inhibition.

2.3. Animals

Male Swiss mice (*Mus musculus*) weighing between 25 and 30 g were used. The animals were obtained from the central animal house of Piauí Federal University and were housed in cages with free access to water and food. They were kept in controlled conditions with constant temperature (25 ± 1 °C) and a 12/12 h light/dark cycle. All experimental procedures were performed in accordance with the guidelines of the Institutional Animal Ethics Committee (protocol N.037/15).

2.4. Induction of ulcerative colitis (UC)

UC was induced according to method described by [3] with slight modifications. The mice were divided into five groups (5–7 animals): saline group (SAL: negative control), acetic acid group (positive control) and experimental groups that were treated with the different doses of LPPp (10, 20 and 40 mg/kg). These doses were the same used by [9] to investigate the anti-inflammatory activity of LPPp. Before the experiment, the animals were starved for 14–15 h. After this period, colitis was induced by single intracolonic instillation of 200 μl of 6% acetic acid solution. The negative control group received an equivalent volume of saline solution (0.9%). Animals were treated with LPPp (10, 20 or 40 mg/kg i.p.) 1 h before and 17 h after acetic acid injection. Eighteen hours after instillation, all animals were euthanized and 5 cm of the colon was excised and weighed to determinate the wet weight. The results were expressed in g/5 cm of colon. Additionally, the colons were evaluated for macroscopic and microscopic lesion scores, myeloperoxidase (MPO) activity, IL1-β levels and concentrations of glutathione (GSH), malondialdehyde (MDA), superoxide dismutase (SOD) and nitrate and nitrite (NO_3/NO_2) in inflamed colon tissue.

2.5. Macroscopic lesion scores

The severity of macroscopic lesions was determined by the scoring system described by [17]. The criteria were: no damage (score 0); focal hyperemia, no ulcers (score 1); ulceration without hyperemia or bowel wall thickening (score 2); ulceration with inflammation at one site (score 3); ≥2 sites of ulceration and inflammation (score 4); ≥2 major sites of ulceration and inflammation or one site of ulceration extending > 1 cm along the intestinal length (score 5); and for damage covering > 2 cm of the intestinal length, score is increased by 1 for each additional centimeter of involvement (scores of 6–10).

2.6. Microscopic lesion scores

For histopathological evaluation, colon samples were fixed in 10% buffered formalin for 24 h. After that, they were washed, dehydrated in alcohol, cleared in xylene and embedded in paraffin. Then the material was sectioned and stained with hematoxylin and eosin. The slides were evaluated (single person, double-blind and randomized analysis) according to the technique described by [18]. Criteria for microscopic intestinal lesion scoring were: loss of mucosal architecture (score 0–3), cellular infiltration (score 0–3), muscle thickening (score 0–3), crypt abscess (score 0–1) and goblet cell depletion (score 0–1).

2.7. Determination of MPO activity, IL-1β and NO_3/NO_2 levels

The level of MPO, a marker for neutrophil infiltration, was estimated using the method described by [19]. For the measurement of the interleukin IL-1β levels, the sandwich Enzyme-Linked Immunosorbent Assay (ELISA) was used according to the manufacturer's recommendations. The results were expressed as picograms per milliliter of homogenate (pg/ml) [9]. The Griess method was used to measure the concentrations of nitrate and nitrite [20]. The results were expressed as micromoles (μM) of nitrite.

2.8. Measurement of colonic SOD, GSH and MDA concentrations

Small samples of colon isolated from the animals with and without colitis were cleared and processed for estimation of the SOD, GSH and MDA concentrations. SOD and GSH are important for endogenous defense against the peroxidative destruction of cell membranes. SOD activity was measured by the protocol describe by [21] and GSH by the method of [22]. The MDA content is an indicator of lipid peroxidation and was determined by the method describe by [23].

2.9. Statistical analysis

The results were expressed as means ± SEM of 5–7 animals. Statistical significance of differences between the groups was determined by one-way analysis of variance (ANOVA) followed by the Student–Newman–Keuls test. Histological scores were assessed by the nonparametric Kruskal − Wallis test followed by Dunn's multiple-comparison test. For all tests, values of $p < 0.05$ were considered significant. Statistical tests were performed with Graphpad Prism (version 5.0).

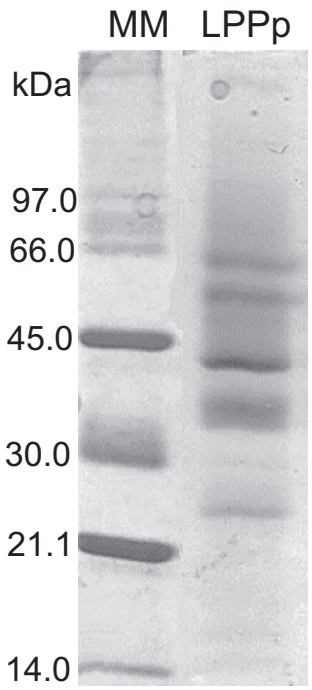

Fig. 1. Polyacrylamide gel (12.5%) electrophoresis of LPPp. Ten micrograms of proteins was applied in each well. Molecular weight markers (MW) were as follows: phosphorylase (97.0 kDa); albumin (66.0 kDa); ovalbumin (45.0 kDa); carbonic anhydrase (30.0 kDa); trypsin inhibitor (20.1 kDa); and lactalbumin (14.4 kDa).

3. Results

3.1. Characterization of LPPp fraction

Electrophoresis revealed that LPPp possesses proteins with molecular weight ranging from 14.0 to 66.0 kDa, with an intense band of around 45.0 kDa (Fig. 1). The colorimetric assay showed the presence of cysteine proteinases (0.229 ± 0.059 UA/μg of protein, at pH 5.0), serine proteinases (0.062 ± 0.005 UA/μg of protein, at pH 10.0) and metalloproteinases (0.100 ± 0.029 UA/μg of protein, at pH 10.0). LPPp inhibited the proteolytic activity of papain (78 ± 3%) and trypsin (55 ± 2%), indicating the presence of cysteine and serine proteinase inhibitors, respectively. The presence of chitinolytic enzymes was confirmed using chitin azure as substrate and the highest chitinolytic activity was seen at pH 6.0 (0.057 ± 0.009 UA/μg of protein) and 10.0. (0.092 ± 0.017 UA/μg of protein) (Table 1). These data corroborate previous findings [9,10] and reveal that LPPp is a well-defined fraction containing proteinases, chitinases and proteinase inhibitors.

Table 1
Biochemical characterization of latex proteins from *Plumeria pudica* (LPPp).

Enzymatic activity	UA/μg of protein	pH
Cysteine proteinases	0.229 ± 0.059	5.0
Serine proteinases	0.062 ± 0.005	10.0
Metalloproteinase	0.100 ± 0.029	10.0
Chitinases	0.057 ± 0.009	6.0
	0.092 ± 0.017	10.0

Inhibitory activity	Percentage of inhibition	pH
Papain	78 ± 3	5.0
Trypsin	55 ± 2	5.0

Samples of 1 mg/ml of LPPp were used for detection of proteinases, chitinases and proteinase inhibitors. Values represent mean ± S.E.M. of four repetitions.

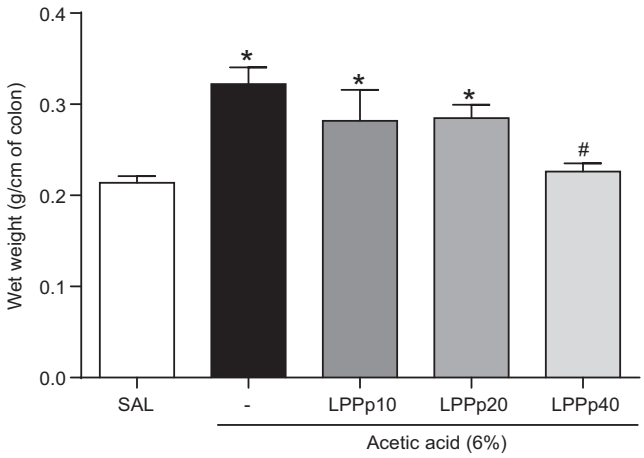

Fig. 2. Effect of latex proteins from *Plumeria pudica* (LPPp) on wet weight in acetic acid-induced colitis in mice. Animals were treated with LPPp (10, 20 or 40 mg/kg) intraperitoneally. They were euthanized and samples of colon tissue (5 cm) were removed, and the wet weight was recorded. The results are expressed as mean ± SEM of 5–7 animals per group. * indicates statistical difference compared with the saline group; # indicates statistical difference compared with the acetic acid group (ANOVA followed by Student-Newman-Keuls test, $p < 0.05$).

3.2. Effect of LPPp on wet weight

The intracolonic administration of acetic acid induced a significant increase in the wet colon weight (0.32 ± 0.02 g/5 cm) compared to the saline group (0.21 ± 0.01 g/5 cm) (Fig. 2). The treatments with LPPp at 10 mg/kg (0.28 ± 0.03 g/5 cm) and 20 mg/kg (0.28 ± 0.01 g/5 cm) were not effective in preventing the increase in wet weight of animals compared to the acetic acid treatment ($p > 0.05$). However, 40 mg/kg LPPp (0.22 ± 0.01 g/5 cm) significantly inhibited the increase in the wet colon weight ($p < 0.05$). No significant differences were observed between the saline group and the group treated with 40 mg/kg of LPPp ($p > 0.05$).

3.3. Effect of LPPp on macroscopic intestinal damage

Macroscopic evaluation of the intestines revealed that the administration of acetic acid in the colon promoted intense tissue injury (16.86 ± 2.19) compared to the saline group (0.33 ± 0.23) (Fig. 3). Animals receiving acetic acid showed intense hyperemia and formation of ulcers in almost all the tissue removed for analysis, whereas the colon material of the animals that received saline had normal appearance without inflammation (Fig. 3). The treatment with different doses of LPPp caused less severe mucosal damage (Fig. 3A). Although treatment of animals with LPPp at 10 mg/kg (13.86 ± 3.40) and 20 mg/kg (12.67 ± 0.97) exhibited a tendency to reduce macroscopic lesion scores (Fig. 3B), significant reduction was observed only in mice receiving 40 mg/kg (8.20 ± 1.47). The effect of LPPp did not lead to lesion scores similar to those of the saline group ($p < 0.05$). Thus, since the dose of 40 mg/kg of LPPp presented the best performance in these evaluations, we chose this dose to investigate further parameters associated with acetic acid-induced colitis.

3.4. Effect of LPPp on microscopic intestinal damage

Tissue samples of the colons of the animals were analyzed by histology (Fig. 4). Acetic acid-induced colitis promoted changes in histological findings, mainly characterized by loss of mucosal architecture, intense inflammatory cell infiltration, muscle thickening, increment in

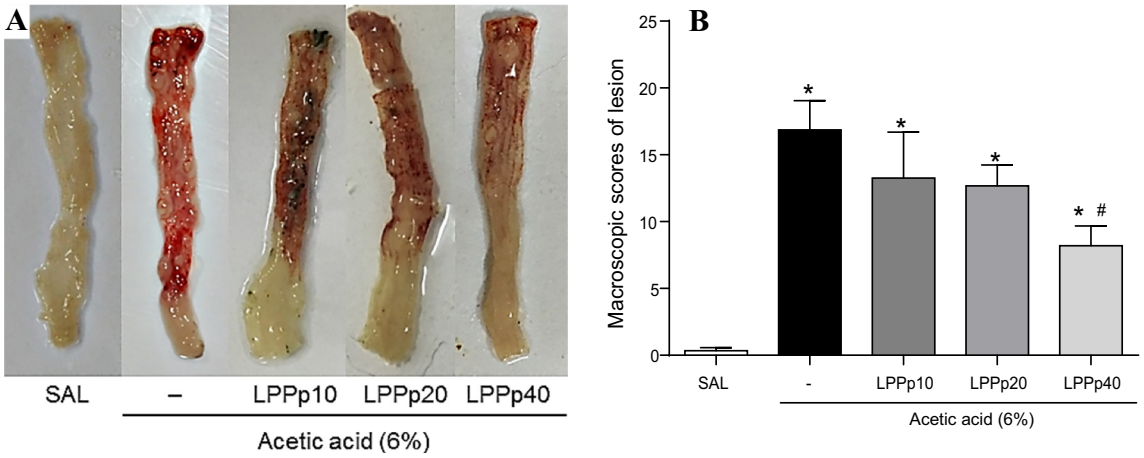

Fig. 3. Effect of latex proteins from *Plumeria pudica* (LPPp) on macroscopic intestinal damage in acetic acid-induced colitis in mice. Animals were treated with LPPp (10, 20 or 40 mg/kg) intraperitoneally. Samples of colon tissue (5 cm) were removed (A) and macroscopic lesion scores were assigned (B). The results are expressed as mean ± SEM of 5–7 animals per group. Pictures of the colons in A are representative samples and data from every animal are included in B. * indicates statistical difference compared with the saline group; # indicates statistical difference compared with the acetic acid group (ANOVA followed by Student-Newman-Keuls test, $p < 0.05$).

crypt abscess and depletion of goblet cells (Table 2). The histological evaluation of mice treated with LPPp 40 mg/kg showed a protective effect against acetic acid-induced colitis (Fig. 4C, Table 2). The colons of mice treated with LPPp (40 mg/kg) presented significant reduction in the inflammatory response, with moderate loss of epithelial cells and minimal inflammatory infiltration into the colonic tissue. This resulted in decreased microscopic damage score and reduction of 45.45% in the total damage score compared with the acetic acid group.

3.5. Effect of LPPp on MPO activity, IL-1β level and nitric oxide concentration

Fig. 5A shows that the intracolonic instillation of acetic acid induced a significant increase of MPO activity (75.85 ± 7.52 UMPO/mg tissue) in the colonic tissue of animals compared to the saline group (2.61 ± 0.75 UMPO/mg tissue). The mice treated with LPPp at 40 mg/kg presented significant reduction in the levels of MPO activity (4.28 ± 1.412 UMPO/mg tissue) compared to the acetic acid group. Moreover, a significant increase in the concentrations of IL-1β was observed in animals receiving only acetic acid (3586 ± 70.25 pg/ml) compared to saline (1794 ± 208.6 pg/ml) (Fig. 5B). Mice treated with LPPp at 40 mg/kg presented significantly lower IL-1β values (1702 ± 150.7 pg/ml) in colonic tissue than those in the acetic acid group ($p < 0.05$). Additionally, the acetic acid group showed a higher level of NO_3/NO_2 in colon tissue (1.77 ± 0.07 μM) compared with the saline group (0.51 ± 0.19 μM) (Fig. 5C). However, the treatment with 40 mg/kg of LPPp did not reduce significantly the levels of nitrate and nitrite (1.41 ± 0.03 μM) ($p > 0.05$).

3.6. Effect of LPPp on MDA, GSH and SOD

Data about oxidative stress markers measured in samples of the colons of mice are presented in Fig. 6. The treatment with LPPp at 40 mg/kg promoted a significant reduction in MDA levels (125.8 ± 10.32 nmol/g tissue) in the colon of the animals compared to the acetic acid group animals (235.2 ± 17.57 nmol/g tissue) (Fig. 6A), while the value of MDA in the saline group was 119.6 ± 18.07 nmol/g tissue. In contrast, the intracolonic administration of acetic acid significantly decreased the concentration of GSH (23.99 ± 4 μg/g tissue) compared to saline (95.20 ± 13.61 μg/g tissue) (Fig. 6B). The treatment with LPPp at 40 mg/kg significantly avoided the consumption of GSH (96.95 ± 19.76 μg/g tissue). In parallel, colitis induced by acetic acid led to higher activity of superoxide dismutase in the colon tissue (6.725 ± 0.2477 UA/mg of protein) compared to the activity in the saline group (4.21 ± 0.08534 UA/mg of protein). The LPPp 40 mg/kg treatment significantly reduced the SOD level (4.87 ± 0.6958 UA/mg of protein) (Fig. 6C). No significant differences were observed in MDA, GSH and SOD between the saline group and the group treated with 40 mg/kg of LPPp ($p > 0.05$).

4. Discussion

In the present work, we evaluated the protective effect of LPPp in acetic acid-induced colitis in mice. This study emerged from our previous observation that LPPp reduced inflammatory and antioxidative parameters in different animal experimental models of inflammation [9,10]. Here, our results show that LPPp treatment (40 mg/kg) had a protective effect on acetic acid-induced colitis by reducing the wet weight and macroscopic and microscopic scores of intestinal lesions. LPPp treatment returned MPO and SOD activities and IL-1β, GSH and MDA concentrations to nearly normal, which contributed to the reduction of inflammation and oxidative stress in the colon.

The experimental model of ulcerative colitis (UC) was used since inflammatory response plays a critical role in the pathogenesis of acetic acid-induced colitis [24]. Transrectal administration of acetic acid produces acute and non-transmural colonic inflammation, which is characterized by intense leukocyte infiltration in the intestinal tissue, overproduction of inflammatory mediators, intense tissue oxidative stress, and extensive necrosis of the mucosal and submucosal layers, followed by intense ulceration [25,26]. Thus, since UC is characterized as an inflammatory disease, molecules presenting anti-inflammatory properties have been tested for treatment of its symptoms [6].

During colitis, the wet weight of the inflamed colon tissue is considered a reliable and sensitive indicator of the severity and extent of the inflammatory response [27]. In addition, acetic acid-induced colitis promotes significant macroscopic changes, characterized by intense hyperemia and formation of ulcers, with inflammation traversing practically the entire affected region [28]. Our results showed that the treatment with LPPp was able to significantly decrease the wet weight of the colon of the mice and the severity of the inflammatory response, as reflected in the lower macroscopic lesion scores.

Additionally, macroscopic tissue changes are accompanied by alterations in microscopic parameters of lesions during induced colitis [29]. The main histological features observed in UC are described as epithelial loss, loss of goblet cells and intense infiltration of inflammatory cells from colonic mucosa, especially neutrophils

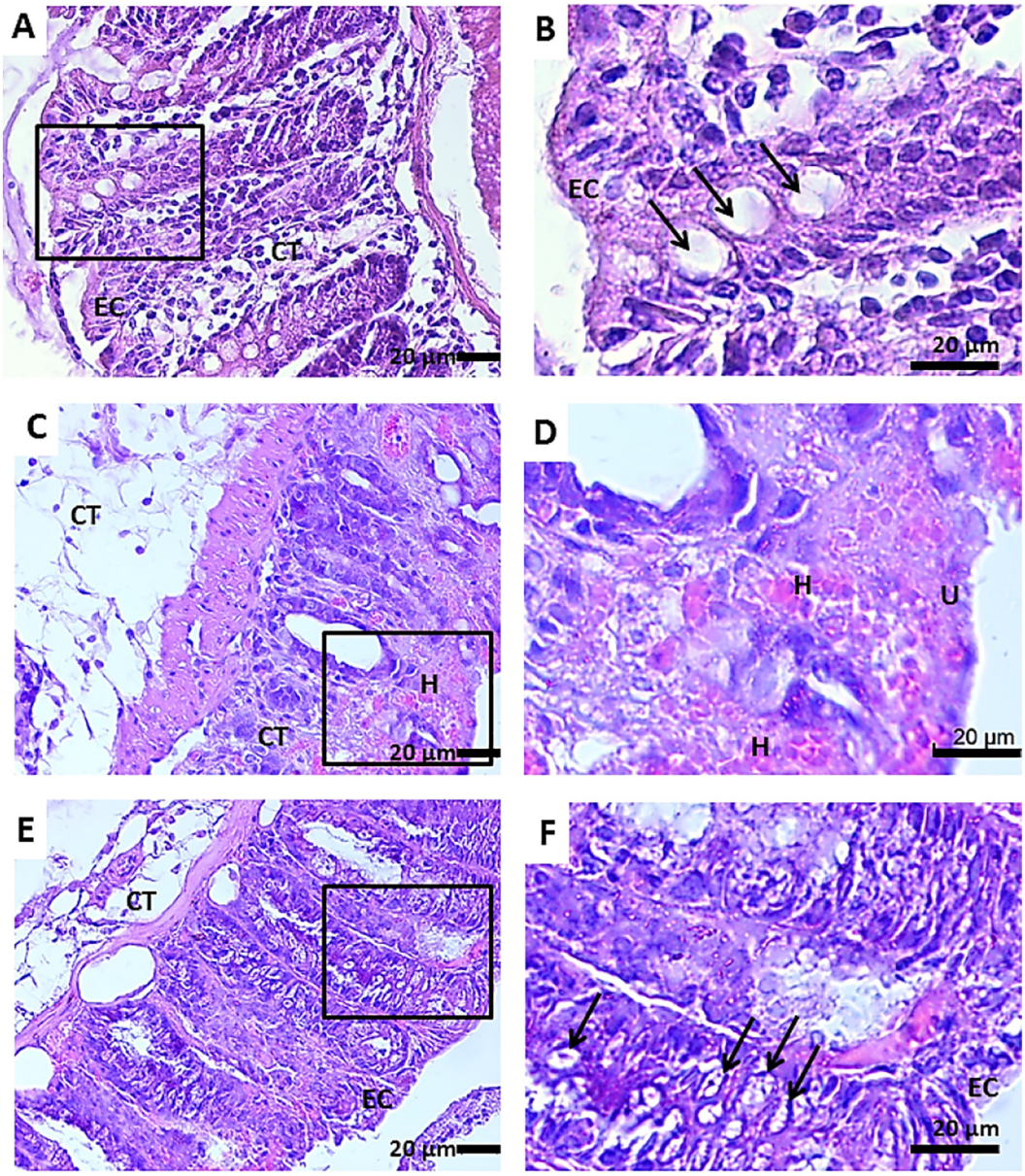

Fig. 4. Effect of latex proteins from *Plumeria pudica* (LPPp) on microscopic intestinal damage in acetic acid-induced colitis in mice. (A) and (B) Saline group, showing normal appearance of mucosa (connective tissue, CT and epithelial cells, EC), with goblet cells (arrows). (C) and (D) Acetic acid group showing extensive degradation of the mucosa, with hemorrhage (H) and ulceration (U). (E) and (F) LPPp (40 mg/kg) group showing prevention of mucosal damage, with preservation of EC and goblet cells (arrows). Images B, D and F are enlargements of boxes in A, C and E, respectively. Pictures are representative samples of tissues from every animal.

Table 2
Effect of latex proteins from *Plumeria pudica* (LPPp) on microscopic intestinal damage in acetic acid-induced colitis in mice.

Criteria	Median score		
	Saline	Acetic acid	LPPp 40 mg/kg
Loss mucosal architecture	0 (0–0)	3 (3–3)*	1 (1–2)#
Cellular infiltration	0 (0–0)	3 (2–3)*	1 (1–1)#
Muscle thickening	0 (0–0)	3 (3–3)*	2 (1–2)
Crypt abscess	0 (0–0)	1 (1–1)*	1 (0–1)#
Goblet cell depletion	0 (0–0)	1 (1–1)*	0 (0–0)#
Total scores of damage	0 (0–0)	11 (10 − 11)*	5 (4–6)#

Score for histological damage expressed as mean ± S.E.M. (n = 5–7).
* $p < 0.05$ versus saline group.
# $p < 0.05$ versus acetic acid group (Kruskal-Wallis non-parametric test and Dunn's test were used for multiples comparisons of histological analyses).

[7,28,30]. A similar pattern was verified in the control (untreated) animals evaluated in the present study. However, the histological changes observed were significantly lower in the group treated with LPPp, indicating that the treatment led to less tissue damage.

Although the etiology of UC is not completely understood, evidence indicates that increase of pro-inflammatory cytokines, such as IL-1β, elevates local inflammation and promotes tissue damage with pathogenic relevance in the development of the disease [4]. In agreement, the animals treated with LPPp presented significantly lower levels of IL-1β than those in the colitis group. The ability of LPPp to diminish the levels of pro-inflammatory cytokines (IL-1β and TNF-α) during inflammation was previously documented [9], in line with our findings.

IL-1β production is closely related to leukocyte migration. This cytokine is produced by resident macrophages, monocytes and dendritic cells, and stimulates adhesion and transmigration of neutrophils to the focus of inflammation [3]. Here, we assessed neutrophil infiltration in colonic tissue by measuring the activity MPO, an enzyme found in

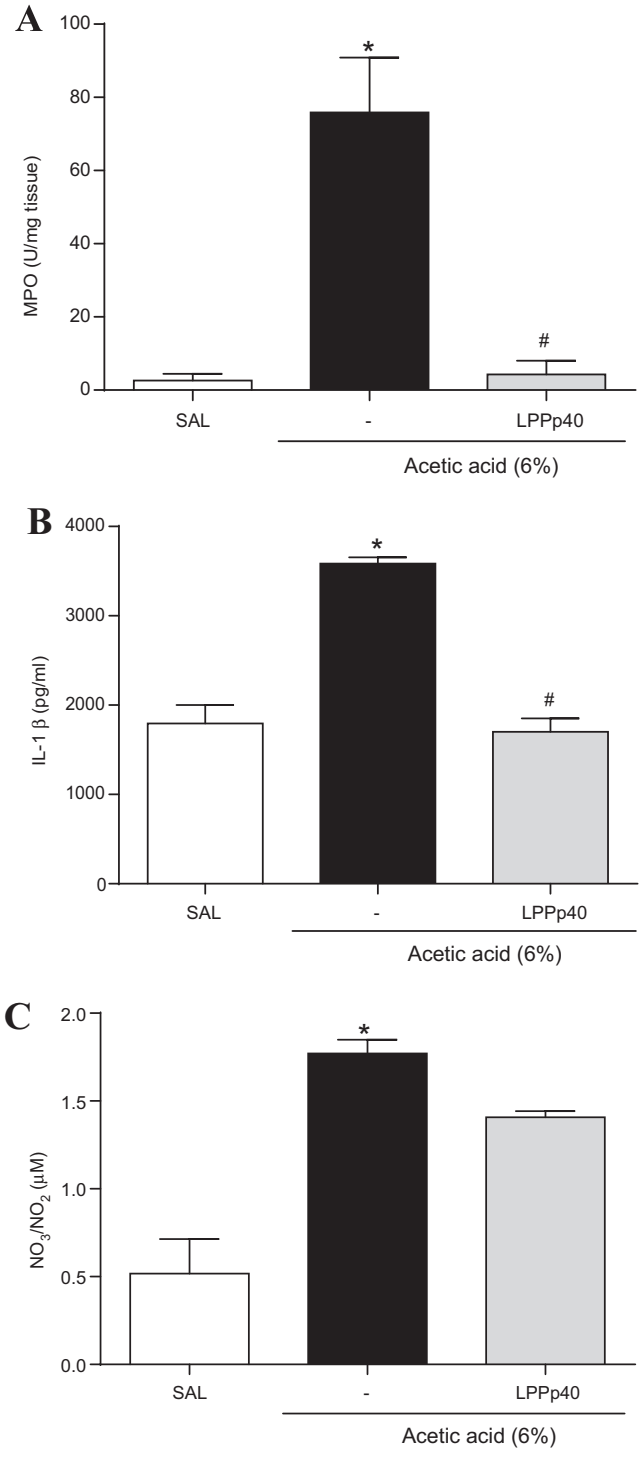

Fig. 5. Effect of latex proteins from *Plumeria pudica* (LPPp) on myeloperoxidase activity (A), cytokine IL-1β level (B), and concentration of nitric oxide (NO_3/NO_2) (C) in acetic acid-induced colitis in mice. The results are expressed as mean ± SEM of 5–7 animals per group. * indicates statistical difference compared with the saline group; # indicates statistical difference compared with the acetic acid group (ANOVA followed by Student-Newman-Keuls test, $p < 0.05$).

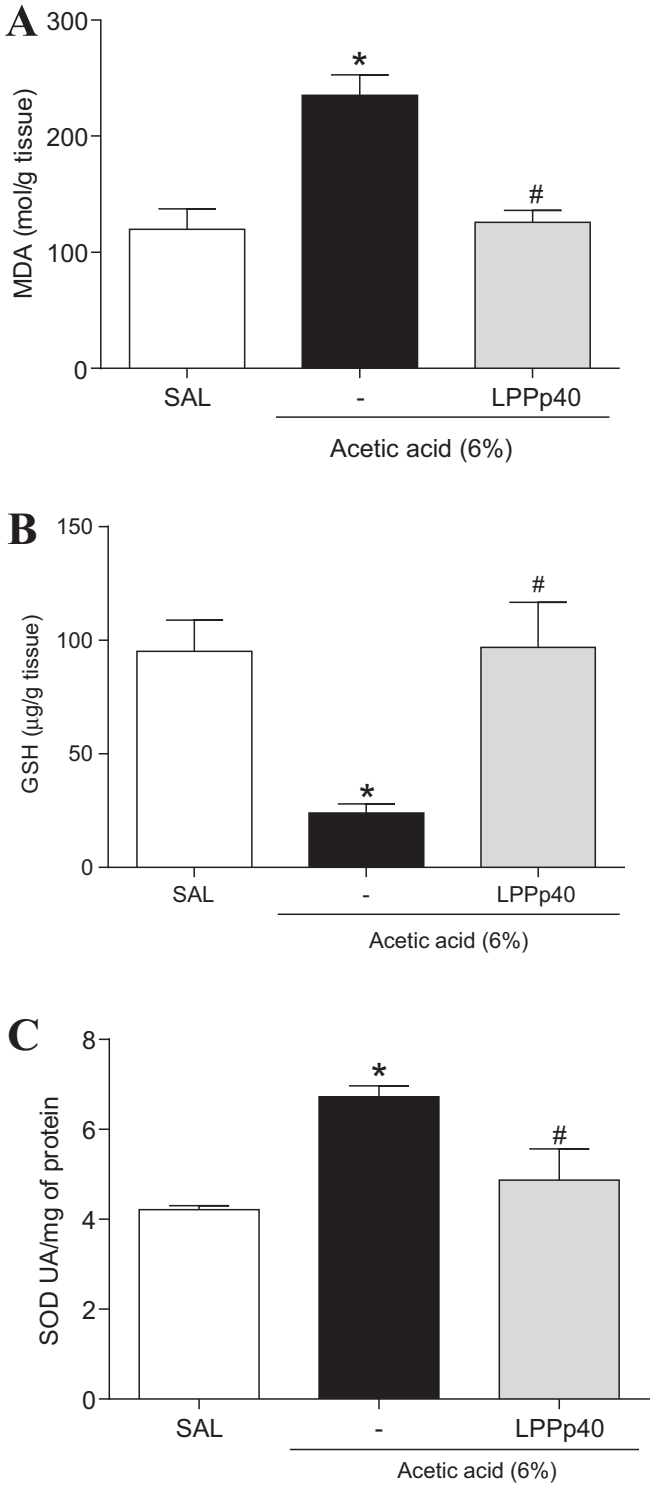

Fig. 6. Effect of latex proteins from *Plumeria pudica* (LPPp) on the levels of malondialdehyde (MDA) (A), glutathione (GSH) (B) and superoxide dismutase activity (SOD) (C) in acetic acid-induced colitis in mice. Samples of colon tissue were removed and the MDA and GSH levels were evaluated. The results are expressed as mean ± SEM of 5–7 animals per group. * indicates statistical difference compared with the saline group; # indicates statistical difference compared with the acetic acid group (ANOVA followed by Student-Newman-Keuls test, $p < 0.05$).

azurophilic granules of neutrophils [27]. High MPO activity reveals intense infiltration of neutrophils, which may contribute to significant tissue damage [29]. In our study, LPPp-treated animals showed significant reduction in MPO activity compared to untreated animals. These data were consistent with the histological findings, that LPPp reduced the gross lesions in the colon. Besides this, the findings for MPO activity corroborate those of [9], in which LPPp significantly reduced the MPO activity induced by the administration of carrageenan in mouse paws. On the other hand, our results demonstrated that LPPp

did not reduce tissue NO_3/NO_2 ratios. High levels of NO are toxic and can damage the tissue directly by peroxynitrite formation after reaction with superoxide [31]. This information indicates that LPPp's effects may not occur via inhibition of peroxynitrite production.

Activated neutrophils can produce reactive nitrogen and oxygen species within the intestinal mucosa, which increases oxidative stress and plays a significant role in the development of UC [32]. During experimental colitis, excessive production of reactive oxygen species in mucosal cells can initiate a cascade of inflammatory events that directly and indirectly damage the intestinal epithelial cells, which disrupts the integrity of the intestinal mucosa barrier [33]. Thus, the level markers of oxidative stress (MDA, GSH and SOD) were evaluated.

MDA is a product of the lipid peroxidation that takes place as a consequence of colonic insult [29]. The tissue increment of MDA levels causes cross-linking of protein and nucleic acid molecules, causing cytotoxicity [6,34]. On the other hand, depletion of tissue GSH has been implicated in the development of UC [35]. These studies have demonstrated that colitis is associated with increased MDA levels and GSH deficiency. Treatment of animals with LPPp during experimental colitis prevented MDA and GSH alterations induced by acetic acid, returning their levels to near normal. We expected LPPp treatment not to interfere in overall values of GSH and MDA, since LPPp previously inhibited these alterations when the inflammatory process was induced [10].

Superoxide dismutase is an enzyme that catalyzes the degradation of reactive oxygen species (ROS) to oxygen and hydrogen peroxide and prevents oxidation in cells of many tissues. Although SOD is an antioxidant, overexpression of this enzyme conversely produces ROS. Thus a balance between ROS and SOD is important [36]. In this study, the treatment of animals with LPPp significantly reduced the levels of SOD. The effect of LPPp in preventing oxidative stress was an important result observed. Treatment strategies that maintained GSH and MDA concentration and reduced SOD activity suggest that LPPp treatment may decrease the incidence of organ failure.

5. Conclusion

Our results showed that LPPp has a protective effect in acetic acid-induced colitis in mice, through mechanisms that involve the inhibition of inflammatory cell infiltration and reduction of oxidative stress and pro-inflammatory cytokine concentration. These results suggest that LPPp contains molecules with potential applications in the development of novel therapeutic agents against this inflammatory bowel disease. LPPp will be further fractionated by ion-exchange chromatography to obtain sub-fractions that will be investigated for the presence of protective effect in acetic acid-induced colitis in mice. The most effective sub-fraction will be submitted to a new chromatography step and the material obtained will be evaluated for the presence of biological activity. In parallel, investigation will be performed to identify the proteins present in LPPp sub-fractions. The main idea is to identify the protein(s) in LPPp with anti-inflammatory activity, confirm the involvement of proteinases, chitinases and/or proteinase inhibitors of LPPp in this event and shed light on the mechanism of action of LPPp proteins in colitis induced by acetic acid.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

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