Peptide Isolated from Noni Seeds Confers Gastroprotective Effect by Improving Inflammation and Oxidative Stress in Mice

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Abstract

Plant molecules are continuously investigated to prevent and treat inflammatory and ulcerative disorders associated with the gastrointestinal tract, such as gastritis, colitis, mucositis, and ulcer. However, most of the work published is devoted to investigating the therapeutic properties of secondary plant metabolites. This work investigated the gastroprotective activity of a lipid transfer protein isolated from *Morinda citrifolia* L., named $McLTP_1$, when orally administered to mice, from the perspective of its use as a novel peptide-based drug for the prevention and treatment of ulcerative gastric lesions. Pretreatment with $McLTP_1$ at different doses (4, 8, or 16 mg/kg) reduced ethanol-induced gastric lesions ($p < 0.05$) in 40%, 84%, and 88%, respectively. In ethanol-induced gastric lesions, it was demonstrated alterations in levels of GSH (↑100%; $p < 0.05$) and a reduction by 45% in the levels of MDA ($p < 0.05$) after $McLTP_1$ administration (8 mg/kg). $McLTP_1$ showed an anti-inflammatory effect through the modulation of the cytokines IL- 10 (↑33%) and TNF-α (↓54%) and was able to reduce MPO levels (↓95%) in the gastric tissue. Besides, the gastroprotective of $McLTP_1$ also involves the production of nitric oxide. The present findings reveal that $McLTP_1$ has a gastroprotective effect dependent, at least in part, on its anti-inflammatory and antioxidant effects.

1. Introduction

Reactive oxygen species (ROS) are products generated from the metabolism of a healthy cell. They are produced mainly by the mitochondria and have a fundamental role in homeostasis (Forrester et al., 2018). However, when cellular ROS overproduction surpasses intracellular antioxidant capacity, oxidative stress occurs, damaging cells, tissues, and biomolecules (Hassan et al., 2017). Several human sufferings are caused by unbalanced oxidative stress status, including cardiovascular and pulmonary diseases, severe liver injury, and gastric lesions (Kumar et al. 2017).

Oxidative stress is recognized as one of the main contributors to the lesions in gastric disorders such as peptic ulcers, gastroesophageal reflux diseases, and gastritis (Jin-Shui et al. 2008). In acute gastric lesions induced by ethanol, severe oxidative stress is triggered due to gastric mucosa lesion, infiltration of neutrophils, and the release of pro-inflammatory cytokines (Sung et al., 2019). Increases in ethanol concentrations lead to increases in ROS, thus causing cell damage and death.

Many plant extracts and isolated molecules have been studied to treat inflammatory and ulcerative gastrointestinal disorders. Regardless, most investigations focus on secondary plant metabolites' gastroprotective activity, such as saponins, anthraquinones, alkaloids, and flavonoids (Singh et al., 2018; Saha et al., 2019).

Plant peptides are considered a promising therapeutic agent class (Sani et al., 2019). According to Baig et al. (2018), peptide-based drugs are used to treat various diseases, such as diabetes and cancer, along with antimicrobials, antifungals, antivirals, and antioxidants. Thus, peptides have emerged as a

significant therapeutics class, with many natural and synthetic analogs undergoing clinical trials (Lau & Dunn 2018).

Our research group recently isolated a peptide from noni (*Morinda citrifolia* L.; Rubiaceae) seeds (*Mc*LTP$_1$; UniProtKB accession number: C0HJH5) with a diverse range of pharmacological activities when orally administered to mice (Campos et al. 2016). *Mc*LTP$_1$ is a thermostable molecule, resistant to proteolytic degradation by pepsin, trypsin, and chymotrypsin, and has shown antipyretic, anti-inflammatory, antinociceptive, and anti-sepsis effects in mice (Campos et al. 2016, 2017; Souza et al. 2018). The anti-inflammatory action mechanism of *Mc*LTP$_1$ is related to cytokines' modulation, such as TNF-α, which is involved in the pathophysiology of the ethanol-induced gastric lesion (Campos et al., 2016, 2017). Hence, we hypothesized that *Mc*LTP$_1$ could also be explored to treat gastric lesions.

This work aimed to evaluate the gastroprotective effects of *Mc*LTP$_1$ and investigate the mechanisms underlying the observed outcomes from the perspective of its future use as a novel peptide-based drug for treating and preventing ulcerative gastric lesions.

2. Materials And Methods

2.1 Drugs and reagents

Tris (hydroxymethyl) aminomethane, trichloroacetic acid (TCA), thiobarbituric acid, ethanol, indomethacin, Nω-nitro-L-arginine methyl ester hydrochloride (L-NAME), ruthenium red, N-acetyl cysteine (NAC), 5,5'-dithiobis (2-nitrobenzoic acid), glibenclamide, 1 H-[1,2,4]oxadiazolo[4,3-a]quinoxaline-1-one (ODQ), hexadecyltrimethylammonium bromide (CTAB), and ranitidine were obtained from Sigma-Aldrich Co. (St. Louis, MO). Enzyme-linked immunosorbent assay (ELISA) kits for tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), and interleukin-10 (IL-10) quantitation were purchased from R&D Systems (Minneapolis, MN). All other chemicals used were of analytical grade and purchased from local suppliers.

2.2 Purification of McLTP$_1$

*Mc*LTP$_1$ was purified from defatted noni (*M. citrifolia* L.; Rubiaceae) seeds flour according to the experimental procedure reported by Campos *et al.* (2016). The proteins were extracted from defatted noni seeds (0.05M Tris-HCl/0.25M NaCl, pH 8.5, 4°C), and the crude extract was fractioned with trichloroacetic acid (TCA) to 2.5% (w/v) final concentration at 30 min on ice. After, the soluble fraction was centrifuged (10,000 × g for 30 min at 4 ∘C) and supernatant dialyzed, lyophilized, applied to Sephadex G-50 column, and monitored at 280 nm.

After isolation, the purity of *Mc*LTP$_1$ was verified by denaturant sodium dodecyl sulfate-polyacrylamide gel electrophoresis (15% SDS-PAGE) under non-reducing conditions (Laemmli 1970). McLTP1 samples were prepared immediately before use for the biological assays based on total soluble protein concentrations (mg/mL) estimated by Bradford (1976), using bovine serum albumin as standard.

2.3 Animals

Swiss male mice (*Mus musculus*) (20–25 g; n = 8/group) were housed at 25 ± 2°C on a 12-h light/dark cycle and received food and water *ad libitum*. Before evaluating the gastroprotective effects of $McLTP_1$, the mice were fasted for a 12 h period, with free access to 5% glucose in water to prevent hypoglycemia and dehydration events. The experiments are reported in compliance with ARRIVE Guidelines and were performed after approval of the Committee for the Ethical Use of Animals of the Federal University of Ceará (CEUA-UFC Proc. Nº 109/16).

2.4 Gastroprotective activity of $McLTP_1$

2.4.1 $McLTP_1$ effects against ethanol-induced gastric lesions in mice

Mice were treated orally via gavage (*per os*, p.o.) with either $McLTP_1$ (4, 8, or 16 mg/kg), vehicle (NaCl 0.15 M; 10 mL/kg of body weight, b.w.), or N-acetyl cysteine (NAC; 300 mg/kg,), 1 h before ethanol administration, to evaluate the gastroprotective effects of $McLTP_1$. The gastric damage was then induced by absolute ethanol (99.8%), also administered orally via gavage (0.1 mL/10 g, b.w.). One hour after ethanol administration, the animals were euthanized by cervical dislocation under anesthesia by halothane inhalation.

The stomachs were removed and opened along the greater curvature, rinsed with saline, extended, and photographed (Medeiros et al. 2008). The hemorrhagic areas were measured using Image Processing and Analysis in Java (ImageJ). The lesion index was calculated in comparison to the ethanol control group as follows: lesion index = [lesion area (mm^2)/ total area of stomach mucosa (mm^2)] x 100 (Alvarez-Suarez et al. 2011).

2.4.2 Histopathological analysis of the stomachs

Additionally, for histopathological analysis, the stomachs of groups (naïve) vehicle - NaCl 0.15 M; $McLTP1$ 8 mg/kg p.o.; or NAC – 300 mg/kg, p.o.) were fixed in 10% formalin solution and dehydrated in 95% ethanol for 24 hours. Finally, the tissues were embedded in paraffin and sectioned. Sections of 4 μm were then stained with hematoxylin and eosin (H&E) for observation under light microscopy (Olympus, Tokyo, Japan). An experienced pathologist analyzed the sections without access to the experimental groups according to previously described criteria (Laine & Weinstein 1988) with the following parameters: edema (score of 0–4), hemorrhagic damage (score of 0–4), and epithelial cell loss (score of 0–3).

2.4.3 Measurement of malondialdehyde (MDA) and glutathione (GSH) levels in stomach tissue

For MDA analysis, the stomach samples from the different groups (naïve mice; vehicle - NaCl 0.15 M; $McLTP_1$ 8 mg/kg p.o.; or NAC – 300 mg/kg, p.o.) were homogenized in 1.15% KCl (10% w/v). Each

stomach homogenate (250 μL) was added to 1.5 mL of 1% phosphoric acid and 500 μL of 0.6% thiobarbituric acid. This mixture was then stirred and heated in a boiling water bath for 45 min. The preparation was then cooled, followed by the addition of 2 mL of *n*-butanol. This mixture was stirred and centrifuged at 1200 rpm for 10 min, and the absorbance was measured at 535 nm. The results were expressed as μM MDA/mg of tissue (Mihara & Uchiyama 1978).

GSH concentration was measured from stomach samples homogenized in 0.02 M ethylenediaminetetraacetic acid (1 mL/100 mg of tissue), distilled water, and TCA (50%, w/v). After centrifugation at 4°C, 3000 rpm for 15 min, the resulting supernatant was mixed with 800 μL of Tris buffer (0.4 M, pH 8.9), followed by the addition of 20 μL of 0.01 M 5,5'-dithiobis-2-nitrobenzoic acid. The absorbance was measured at 412 nm using a spectrophotometer. The results were expressed as μg GSH/mg of tissue (Sedlak & Lindsay, 1968).

2.4.4 Determination of myeloperoxidase (MPO) enzyme activity

Neutrophil migrations on ethanol-induced gastric lesions were determined by the activity of the enzyme MPO, according to the method described by Bradley et al. (1982). Samples of the gastric mucosa obtained from different experimental groups (naïve mice; vehicle - NaCl 0.15 M; McLTP$_1$ 8 mg/kg p.o.) were homogenized in 50 mM sodium phosphate buffer (pH 6.0) containing 0.5% hexadecyltrimethylammonium (100 mg tissue/1.5 mL) and grounded in a tissue homogenizer. The homogenate was centrifuged for 15 min at 2,500 rpm. The supernatant (10 μL) was then added to a 96-well microplate in duplicate. Subsequently, 200 μL of buffer solution containing o-dianisidine dihydrochloride (16.7 mg), deionized water (90 μL), sodium phosphate buffer (10 μL), and 1% hydrogen peroxide (50 μL) were added to the wells. After 5 min, the absorbance of samples was measured in a microplate reader (450 nm), and the enzymatic activity of MPO was expressed as units of MPO/mg of tissue.

2.4.5 Quantification of IL-1β, TNF-α, and IL-10

The cytokines IL-1β, TNF-α, and IL-10 were measured in supernatants of stomach homogenates of ethanol-induced gastric lesion models using commercial ELISA kits. Stomach samples from groups (naïve mice; vehicle - NaCl 0.15 M; McLTP$_1$ 8 mg/kg p.o.) were homogenized in tissue homogenizer in phosphate-buffered PBS (100 mg tissue/1 mL buffer) and centrifuged (3,000 rpm, 4°C, 10 min). The supernatant (100 μL) was collected and incubated in a 96-well microplate with the capture antibody (24 h, 4 °C) following reaction blockage with bovine serum albumin. The levels of IL-1β, TNF-α, and IL-10 were calculated by standard curves acquired according to the manufacturer's instructions. The results were expressed as pg/mL of gastric homogenates (Tavares-Murta et al., 2008).

2.4.6 Participation of nitric oxide (NO), soluble guanylate cyclase (sGC), potassium channel ATP-sensitive (K$_{ATP}$), prostaglandins (PGs), and capsaicin-sensitive afferent neurons in the gastroprotective effect of McLTP$_1$ on the ethanol-induced gastric lesion model

First, animals were pretreated with vehicle (NaCl 0.15 M; 10 mL/kg, p.o.), L-NAME (20 mg/kg, intraperitoneal, i.p.), ODQ (10 mg/kg, i.p.), glibenclamide (10 mg/kg, i.p.), indomethacin (10 mg/kg, subcutaneous, s.c.), or ruthenium red (3 mg/kg, s.c.). McLTP1 was administered to mice (8 mg/kg, p.o.) one hour after the pretreatment with ODQ, glibenclamide, or indomethacin and 30 minutes after administering L-NAME or ruthenium red (De Alencar et al., 2015 with modifications). The gastric damage induction and the lesion area measurements were performed as described in section 2.4.1.

2.4.7 Effect of $McLTP_1$ on gastric secretion

Mice were pretreated orally with vehicle NaCl 0.15 M (10 mL/kg), ranitidine (50 mg/kg), or $McLTP_1$ (8 mg/kg). After 1 h, animals were anesthetized with ketamine (80 mg/kg, i.p.) and xylazine (10 mg/kg, i.p). The abdomen of the treated animals was opened for the exposition of the stomach, and subsequently, the pylorus ligation was done. Four hours later, the animals were euthanized by cervical dislocation under anesthesia by halothane inhalation. The stomach was opened, and the gastric content was collected using ultrapure water (2 mL) and centrifuged at 1,500 rpm, 20 min, 25°C. Next, the total acidity (mEq [H^+]/mL) was determined by titrating the homogenates with 0.01 M NaOH using a pH meter. Changes in pH were inferred as changes in gastric secretion (Shay 1945).

2.5 Statistical analysis

Shapiro–Wilk test was performed to decide normality. The data with $p > 0.05$ (Shapiro-Wilk normality test) were considered normal and analyzed using a one-way analysis of variance (ANOVA), followed by Tukey's test for multiple comparisons. The nonparametric Kruskal-Wallis comparisons test analyzed non-normal data. The statistical analysis was performed using Graph Pad Prism 6.0 (Graph Pad Software Inc., CA, United States). The letters of the statistics indicate the values show a statistical difference ($p < 0.05$) among the compared groups.

2.6 Ethics Approval of animal use

The animals were treated in compliance with the ethical standards established by the National Guidelines for the Use of Experimental Animals of Brazil and Directive 2010/63/EU of the European Parliament and the Council of the European Union. The experiments were performed after approval of the Committee for the Ethical Use of Animals of the Federal University of Ceará (CEUA-UFC Proc. Nº 109/16).

3. Results

3.1 Gastroprotective effect of $McLTP_1$ on ethanol-induced gastric lesion

Ethanol administration induced intense macroscopic gastric damage in mice (14.57 ± 3.35 of lesion index). The oral treatment with $McLTP_1$ (4, 8, and 16 mg/kg) significantly decreased ($p < 0.05$) the appearance of ethanol-induced lesions with reductions of 40% (8.73 ± 1.76 of lesion index), 84% (2.28 ±

0.75 of lesion index), and 88% (1.73 ± 0.71 of lesion index) of the lesion areas, respectively when compared to the vehicle group. The doses of 8 and 16 mg/kg were not significantly different from each other, as well as they did not differ from the standard drug N-acetylcysteine (300 mg/kg), which reduced gastric lesions by 89% (1.59 ± 0.77 of lesion index) (Fig. 1).

Histological analyses from ethanol-induced gastric lesions in mice of all experimental groups are presented in Fig. 2 (A, B, C, D, E, F, and G). The vehicle group manifested severe gastric damage evidenced by scores of edema: 2 (2–3), epithelial cell loss: 2 (2–3), and hemorrhage: 2 (2–4) in the mucosal layer. Treatment with $McLTP_1$ (8 mg/kg; p.o.) displayed marked gastric mucosal protection ($p < 0.05$) evidenced by mild submucosal edema 1 (0–2), epithelial cell lost 2 (0–2), and absence of hemorrhage 0 (0–0) in the mucosa. NAC (300 mg/kg; p.o.) pretreated group showed no signs of histological damage and was significantly similar to the $McLTP_1$ treated group.

3.2 Effect of McLTP$_1$ on Oxidative Stress and Inflammation

Compared to basal values seen in naïve - non-treated mice (MDA: 22.49 ± 0.64 μM/mg tissue and GSH: 180.5 ± 4.5 μg/mg tissue), ethanol significantly increased MDA gastric tissue levels by 68% (70.69 ± 12.5 μM/mg tissue) and depleted GSH levels by 41% (105.8 ± 13.8 μg/mg tissue). However, pretreatment with $McLTP_1$ (8 mg/kg, p.o.) decreased MDA levels by 45% and increased GSH levels by 100% compared to the vehicle ($p < 0.05$), similar to the results observed for the treatment with NAC (300 mg/kg; p.o.) ($p > 0.05$) (Table I).

Pretreatment of mice with $McLTP_1$ (8 mg/kg; p.o.) significantly reduced ($p < 0.05$) the MPO activity compared with the vehicle group (Fig. 3). Pretreatment of mice with $McLTP_1$ (8 mg/kg; p.o.) also caused a significant decrease in TNF-α levels by 54% and promoted an increase by 33% in IL-10 levels ($p < 0.05$) when compared to the control group. Contrary to that, $McLTP_1$ was unable to reduce the levels of IL-1β significantly (Fig. 4).

3.3 Role of nitric oxide (NO) and evaluation of anti-secretory activity

The gastroprotective effect of $McLTP_1$ was partially reversed by 45% when the animals were pretreated with L-NAME (20 mg/kg; i.p.) ($p < 0.05$). However, pretreatment of mice with ODQ (10 mg/kg; i.p.), glibenclamide (10 mg/kg; i.p.), indomethacin (10 mg/kg; s.c.), or ruthenium red (3 mg/kg; s.c) did not reverse the effects of $McLTP_1$ 8 mg/kg (p.o.) (Fig. 5).

After pyloric ligation, gastric acidity and pH of the vehicle group were 0.004 ± 0.0007 mEq [H⁺]/mL and 2.41 ± 0.08, respectively. Treatment of mice with $McLTP_1$ (8 mg/kg; p.o.) reduced the total acidity by 37.5% (0.025 ± 0.0006 mEq [H⁺]/mL) and increased the pH to 2.61 ± 0.08. As expected, ranitidine (50 mg/kg; p.o.) reduced the total acidity by 35% (0.026 ± 0.0005 mEq [H⁺]/mL) and increased the pH (2.60 ±

0.09). The effect of ranitidine did not differ to that observed for the treatment of mice with $McLTP_1$ ($p >$ 0.05) (Fig. 6).

4. Discussion

Numerous recent studies have considered plants as alternative sources for developing new drugs for treating the gastrointestinal tract's chronic diseases because of their high therapeutic potential and fewer adverse effects (Singh et al. 2018). A plant species rich in bioactive molecules and widely used in traditional medicine is *M. citrifolia L.* (Rubiaceae), popularly known as noni (Assi et al. 2017). Diverse pharmacological activities have been reported for this species, including antidiabetic, antioxidant, anti-inflammatory, gastric ulcer healing, and hepatoprotective. Although over 200 different compounds have been identified in noni (Assi et al. 2017), research on proteins isolated from this plant is still scarce. In this work, we demonstrate for the first time that a protein isolated from noni seeds presents promising therapeutic effects against gastric injuries in mice. It is also the first report of the gastroprotective activity of plant LTPs.

$McLTP_1$ effects were studied after oral administration to mice to provide the first report for future clinical use. Ethanol causes high production of ROS, produces necrotic lesions, and exposes the epithelium to pepsin and hydrochloric acid (Zatorski et al. 2018). Treatment of mice with $McLTP_1$ decreased the gastric lesion index and reduced histopathological alterations caused by ethanol, demonstrating that $McLTP_1$ displays a gastroprotective effect. As observed for the anti-inflammatory activity already investigated for $McLTP_1$ (Campos et al. 2016), there were no significant differences in the effects observed between the treatment of mice with 8 or 16 mg/kg. Besides, the gastroprotective action displayed by the $McLTP_1$ treatment is not different from that presented by the positive control, NAC. Considering one of the most common side-effects of continuous use of anti-inflammatory drugs is the appearance of gastrointestinal injuries, the described gastroprotective effects performed by $McLTP_1$ in this study reinforce the potential use of this protein as a less harmful alternative to treat inflammation, as proposed by Campos et al. (2017).

Alcoholic extracts, secondary metabolites, and polysaccharides have been described as potentially gastroprotective molecules from plants (Diniz et al., 2015; Neto et al., 2017; Sidahmed et al., 2013; Carlotto et al., 2019). However, few studies are still related to the gastroprotective activity of plant proteins or peptides. The advantages of proteins/peptides over molecules from secondary metabolism are mainly because the former has high selectivity, fewer side effects, reduced toxicity, and can be designed to act on vast mechanisms, increasing the possibilities of their application against different diseases. However, a disadvantage associated with proteins/peptides has been their low oral bioavailability due to degradation by gastrointestinal tract enzymes (Bruno et al., 2013). Remarkably, $McLTP_1$ is stable to the main enzymes of the gastrointestinal tract (trypsin, chymotrypsin, and pepsin) (Campos et al. 2016), which enables the application of $McLTP_1$ orally by overcoming the main problem associated with the use of therapeutic proteins/peptides.

Oxidative stress and inflammation are parameters related to the pathophysiology of many diseases. Thus, drugs that modulate ROS production and inflammation can act to treat a diverse set of conditions, including peptic ulcers, gastrointestinal cancers, and inflammatory bowel disease (Bhattachayya et al., 2014). Gastric mucosal damage induced by ethanol has been suggested to be mediated through enhanced oxidative stress (Suzuki et al., 2012; Tamura et al., 2013). Oxidative stress disrupts a subtle oxidant/antioxidant balance, promotes lipid peroxidation and mucosal blood extravasation, and increases infiltration of activated neutrophils (Halliwell, 1994; Asmari et al., 2016). The observed prevention of GSH depletion, combined with L-NAME partial reversion of McLTP$_1$ gastroprotective effect, suggests a potential McLTP$_1$ action mechanism to reduce oxidative stress.

Corroborating with the data presented by Campos et al. (2017), McLTP$_1$ showed a significant reduction of MPO activity, an indirect parameter of neutrophil accumulation in gastric mucosal tissues. Neutrophil release in the inflammatory response leads to increased gastric expression of NF-κB, which stimulates the synthesis of pro-inflammatory cytokines, including tumor necrosis factor-α (Yoo et al., 2018). In contrast, the anti-inflammatory cytokine IL-10 is considered a potent molecule for eliminating inflammatory processes involved in maintaining the homeostasis of the gastric mucosa, including the synthesis of the regulator TNF-α (Lee et al. 2017). The ratio of pro- (TNF-α) and anti-inflammatory (IL-10) cytokines may influence the degree of inflammation, an essential factor for gastric lesions (Kumar et al. 2015). The data presented here reinforces the idea that McLTP$_1$ may exert anti-inflammatory and antioxidant therapeutic effects via the involvement of cytokines, such as TNF-α and IL-10. In contrast, McLTP$_1$ could not act over IL-1β, suggesting McLTP$_1$ selectivity across the tested cytokines.

It is known that inflammatory cells are directly linked to oxidative stress in gastric mucosa injuries, as the ROS generated by neutrophils promotes lipid peroxidation (Raish et al. 2018). Given *that* McLTP$_1$ did not show any direct antioxidant activity, it can be reasoned that the antioxidant defenses elevated by McLTP$_1$ described in this study are partially or entirely due to reducing these inflammatory factors.

It has been known that NO plays an essential role in maintaining gastric mucosal integrity (Antosova et al., 2012) and reduces acid secretion from gastric parietal cells (Lanas, 2008). Previous administration of the NO synthesis inhibitor, L-NAME, reverted the action of McLTP$_1$, suggesting that the gastroprotective activity of this protein may involve the modulation of NO pathways, leading to an increase in the NO levels on the gastric mucosa. Our results also showed that McLTP$_1$ decreased the total acidity of gastric juice in the ligature pylorus model and suggested that the increased pH is indirectly related to NO and the production of GSH in the gastric mucosa.

Pretreatment of mice with ODQ, glibenclamide, indomethacin, or ruthenium red, did not modify the gastroprotective activity of McLTP$_1$. These results indicate that sGC, K$_{ATP}$, PGs, and capsaicin-sensitive sensory receptors are not involved in the effects of McLTP$_1$. Our findings are similar to those described by Pinheiro et al. (2013). Proteins isolated from the latex of *Himatanthus drasticus* showed a

gastroprotective effect against ethanol-induced gastric lesions. They mediated the restoration of GSH and nitrite levels in the mucosa and modulation of the NO/cGMP/KATP pathway.

LTPs are commonly associated with plant defense (Finkina et al. 2016), and they are underestimated from a therapeutic point of view. However, this study showed for the first time an LTP isolated from noni seeds with gastroprotective action and, at the same time, analgesic, anti-inflammatory, and antipyretic effects (Campos et al. 2017). Our work demonstrates the therapeutic potential of plant LTPs, evidencing $McLTP_1$ as a strong candidate for developing a new drug of protein origin to treat gastrointestinal tract disorders.

5. Conclusion

M. citrifolia lipid transfer protein is the first lipid transfer protein with gastroprotective effects studied. Its pharmacological effects are related to modulating inflammation and ROS production. These modes of action of $McLTP_1$ are of significant clinical importance. However, standardized pharmacological safety studies will be necessary to ensure safe use.

Declarations

Author contributions

FCN, HDO, and ROSD conceived the project, designed the experiments, and analyzed the data. FCN, ASC, DCOC, and AXF performed the experiments. FCN, HDO, ROSD, and RSG wrote the manuscript and made critical revisions for intellectual content. PMGS and ROSD analyzed the histopathological slide images. ROR contributed to the English written review. HDO, NMNA, and MHLPS contributed to financial obtaining and support. The authors declare that they have no conflict of interest.

Acknowledgments

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Tables

Table I. Effect of McLTP$_1$ on MDA and GSH levels in the mice gastric mucosa of the ethanol-induced gastric lesion. The gastric damage was induced by absolute ethanol (99.8%), administered orally via gavage (0.1 mL/10 g, b.w.). Data were expressed as the mean ± SD (n = 8). The letters of the statistics indicate the values show a statistical difference (p < 0.05) among the compared groups.

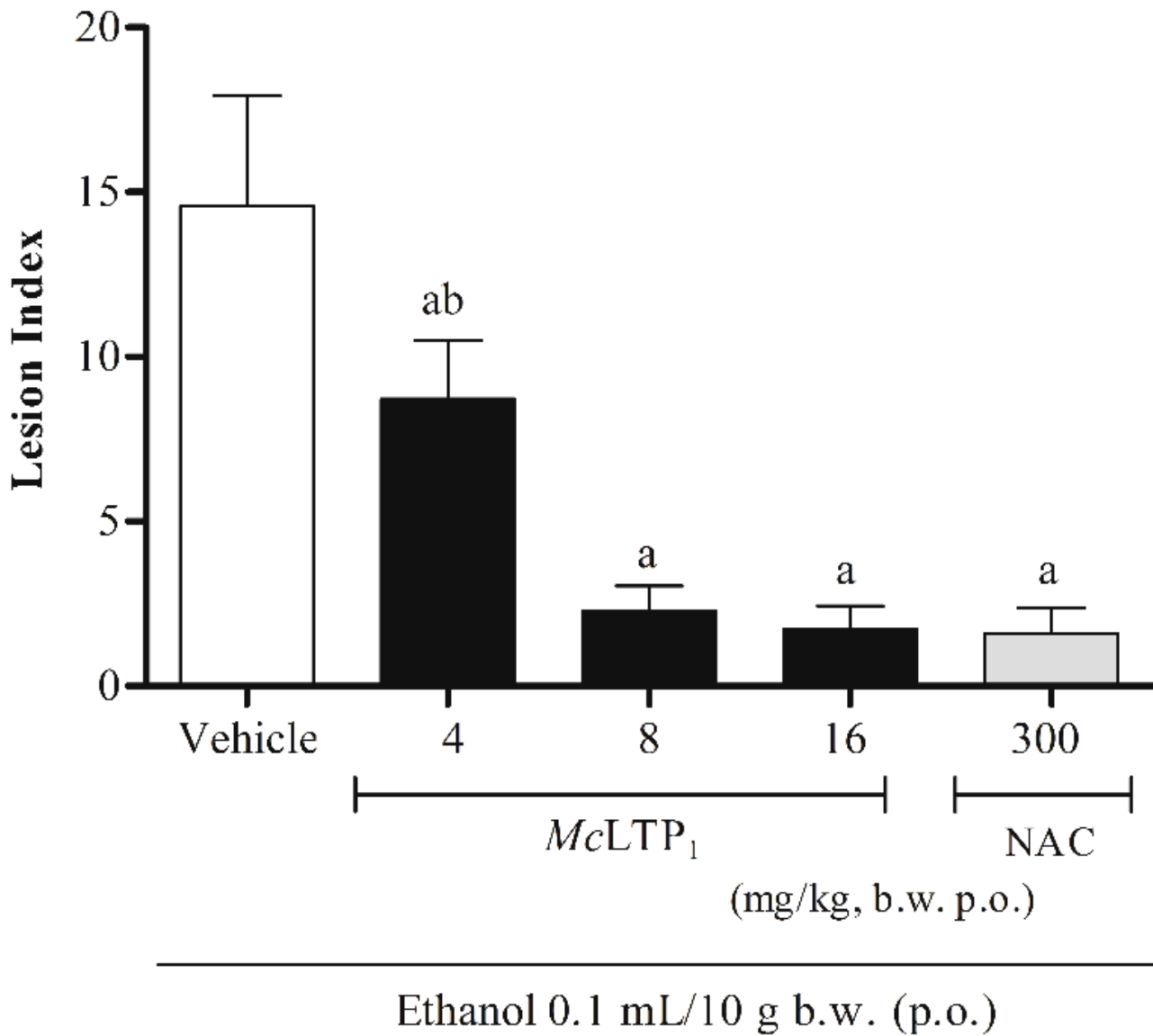| Treatment (n=8)	MDA (μM/mg tissue)	GSH (μg/mg tissue)
Naïve	22.49 ± 0.64 [b]	180.5 ± 4.5 [b]
Vehicle (saline)	70.69 ± 12.5 [a]	105.8 ± 13.8 [a]
*Mc*LTP$_1$ (8 mg/kg, p.o.)	39.8 ±11.6 [ab]	220.2 ± 38.38 [b]
NAC (300 mg/g, p.o.)	38.6 ± 1.9 [ab]	182.6 ± 28.8 [b]

Figures

Figure 1

Gastroprotective effect of $McLTP_1$ on the ethanol-induced gastric lesion. Mice were treated orally (p.o.) with either $McLTP_1$ (4, 8, or 16 mg/kg), vehicle (NaCl 0.15 M; 10 mL/kg of body weight, b.w.), or N-acetyl cysteine (NAC; 300 mg/kg, positive control) 1 h before ethanol administration. Values are expressed as mean ± SD; [a]$p < 0.05$ compared to vehicle and [b]$p < 0.05$ compared to NAC; (one-way ANOVA followed by Tukey's test; n= 8).
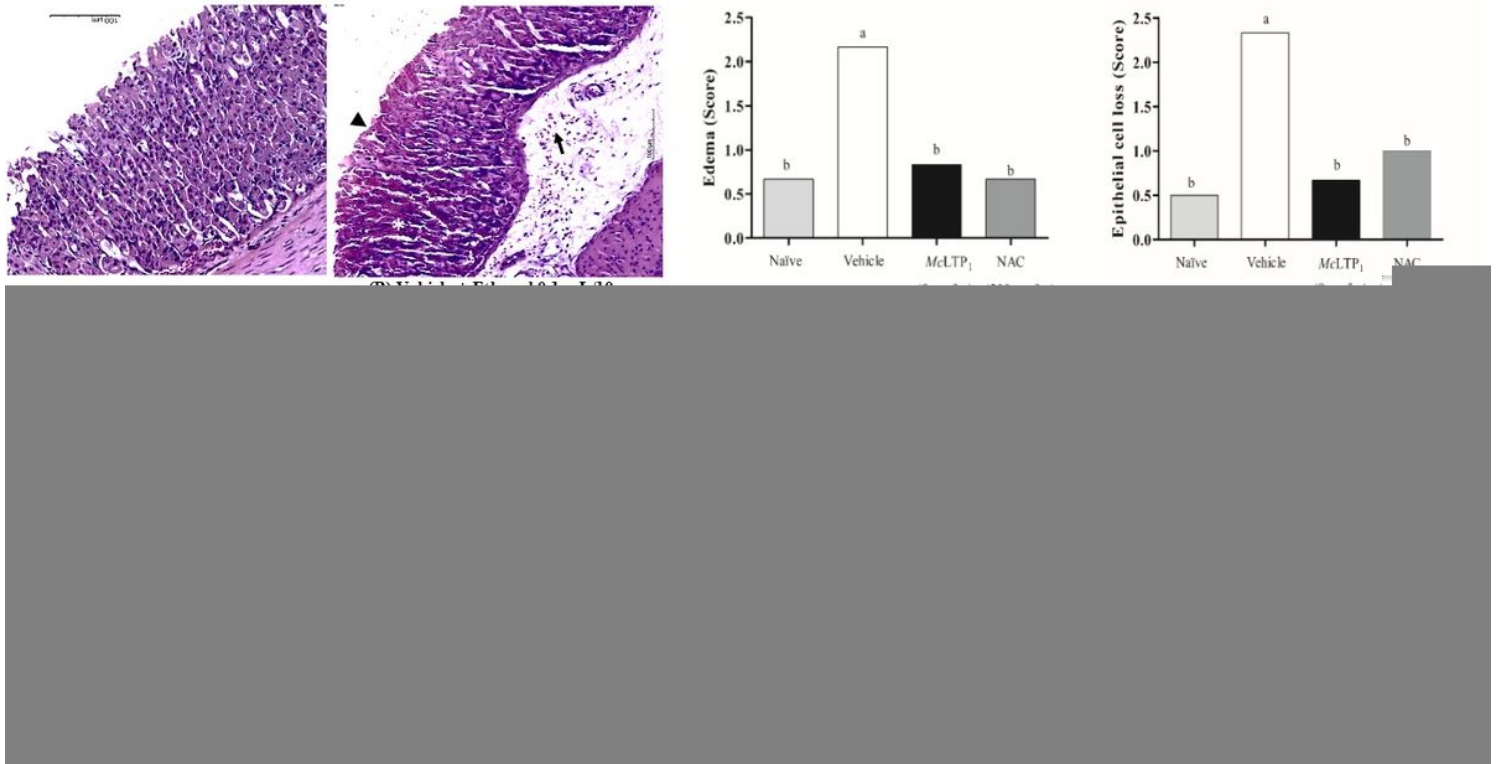

Figure 2

Histopathological alterations in Hematoxylin and Eosin (H&E) stained gastric mucosa. (A) naïve - the normal histological appearance of stomach, (B) vehicle + ethanol − edema (arrow), epithelial cell loss (arrowhead), and hemorrhagic damage (asterisk), (C) pretreatment with $McLTP_1$ 8 mg/kg or (D) NAC 300 mg/kg. (n=8; H & E stain: 20×, scale bar 100 μm). The section was analyzed by the presence or absence of edema (E), epithelial cell loss (F), and hemorrhagic damage (G). Results are presented as medians, and the Kruskal-Wallis nonparametric test, followed by Dunn's test, was used for multiple comparisons of histological analyses (n=8). Significance is represented as [a]$p < 0.05$ *vs* naïve and as [b]$p < 0.05$ *vs.* vehicle.
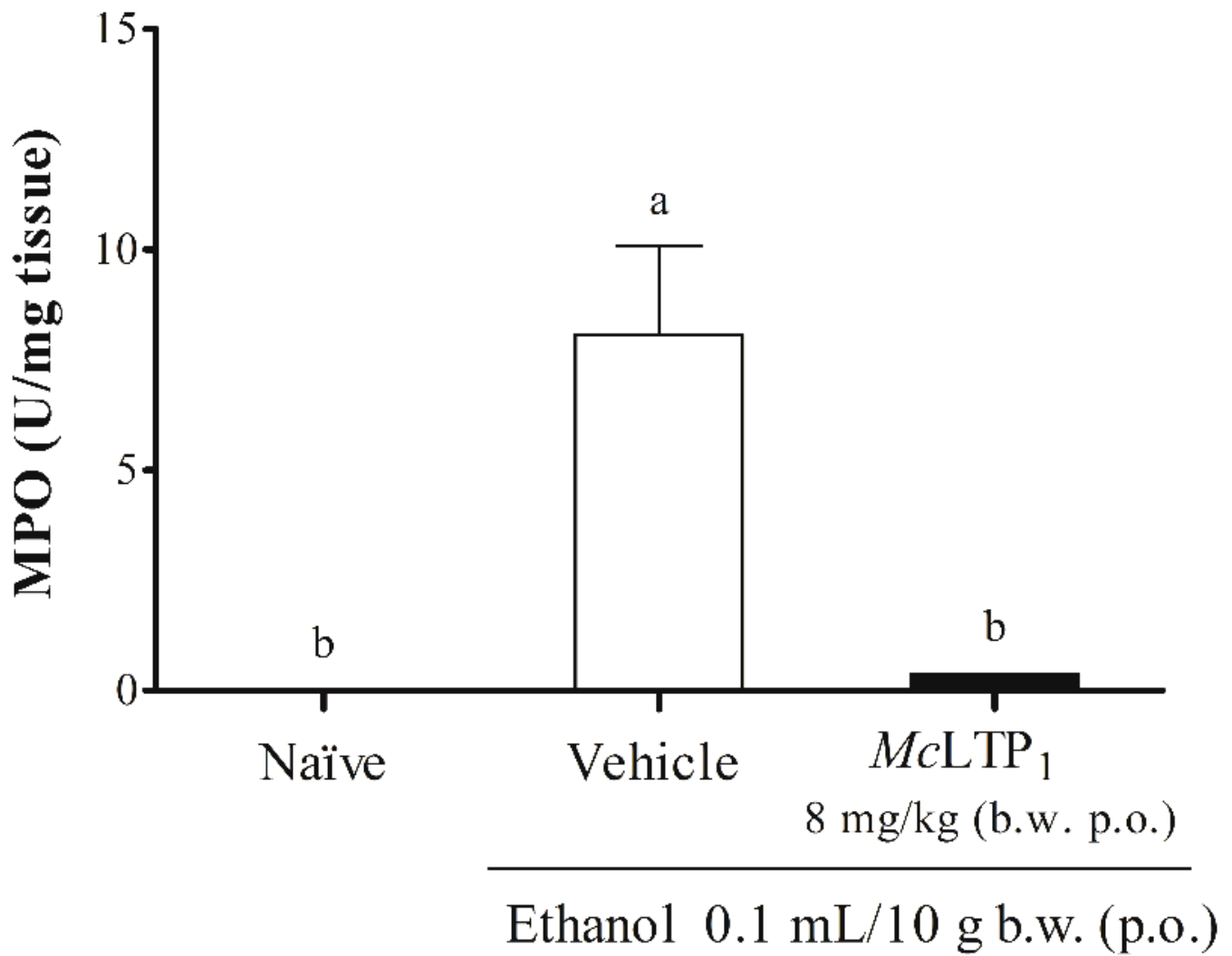
Figure 3

Levels of gastric MPO on ethanol-induced lesions after oral treatment of mice with saline (0.15 M NaCl; 10 mL/kg) or McLTP$_1$ (8 mg/kg), 1h before ethanol administration. Values are expressed as mean ± SD; [a]$p < 0.05$ $vs.$ naïve and [b]$p < 0.05$ $vs.$ vehicle (one-way ANOVA followed by Tukey's test; n= 8).
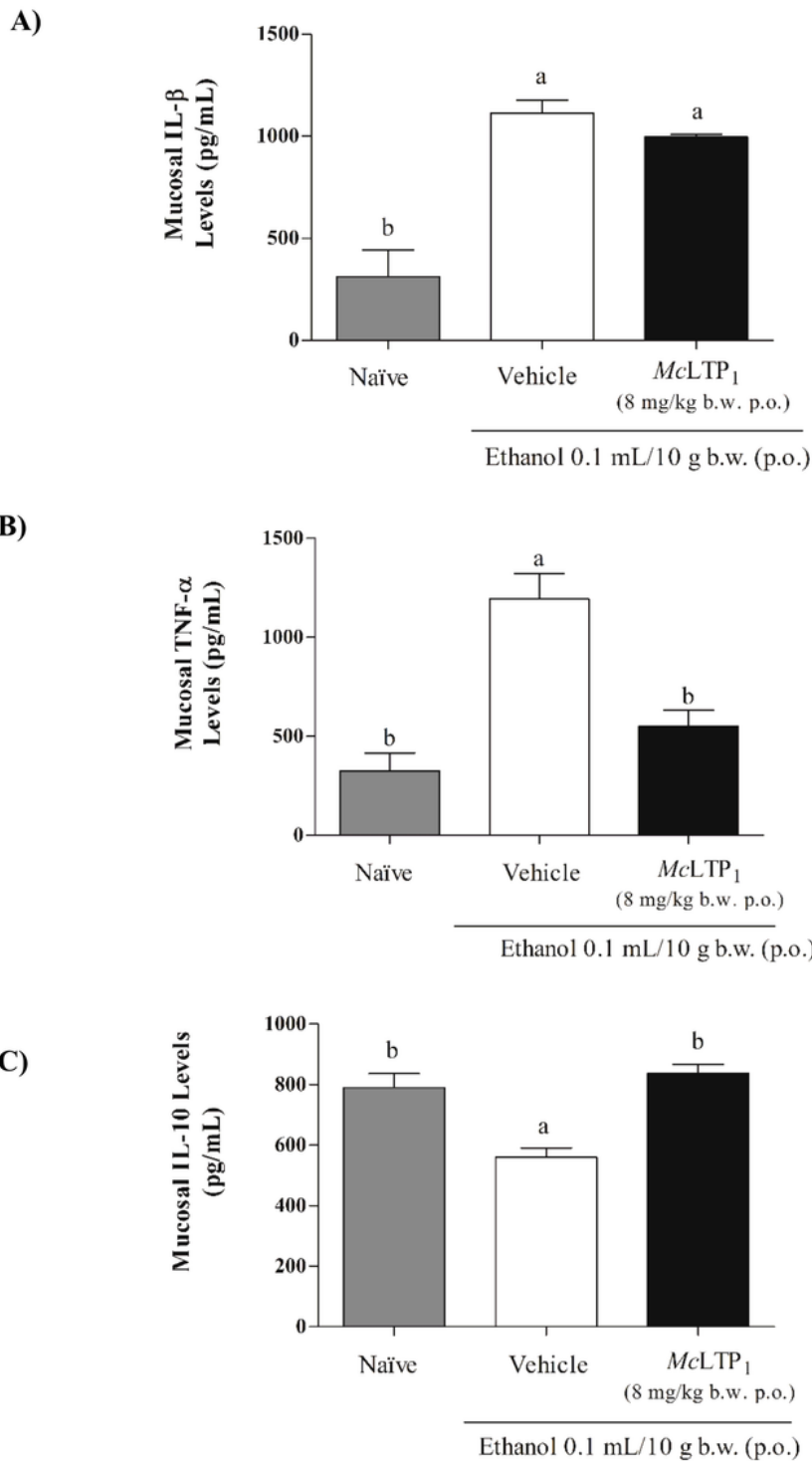
Figure 4

A)

B)

C)

Figure 4

Involvement of interleukin 1β (IL-1β), interleukin 10 (IL-10), and tumor necrosis factor α (TNF-α) on ethanol-induced gastric injury in mice. Levels of cytokines on ethanol-induced gastric lesions after oral administration of saline (0.15 M NaCl, 10 mL/kg) or McLTP$_1$ (8 mg/kg), 1h before ethanol administration. (A) cytokine TNF-α, (B) cytokine IL-10, and (C) cytokine IL-1β. Data are presented as mean ± SD. [a]$p < 0.05$ vs naïve and [b]$p < 0.05$ vs. vehicle (one-way ANOVA followed by Tukey's test; n = 8).
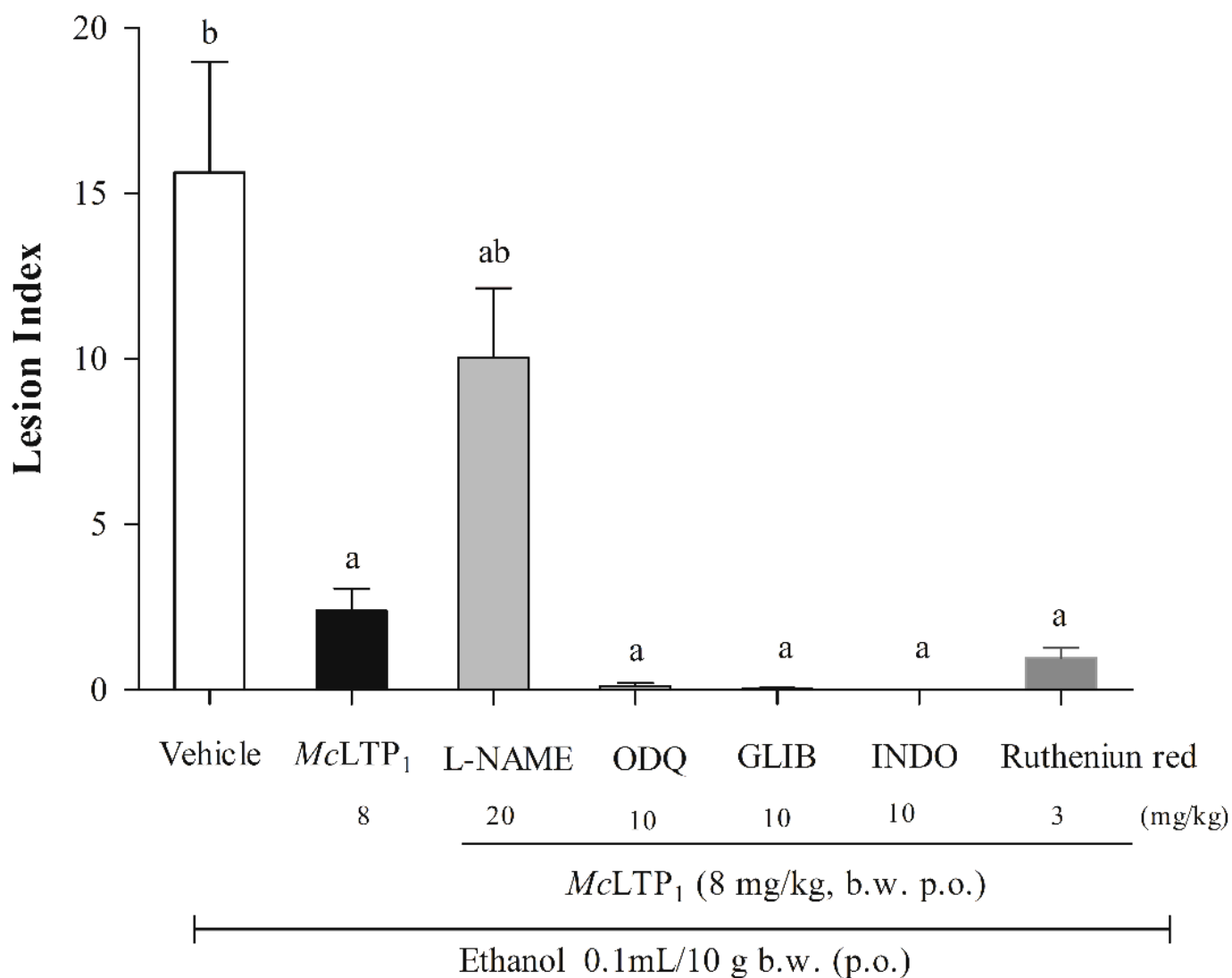
Figure 5

Involvement of nitric oxide, soluble guanylate cyclase (sGC), potassium channel ATP-sensitive (K_{ATP}), prostaglandins, and $TRPV_1$ channels in the gastroprotective effect of McLTP$_1$ 8 mg/kg (p.o.) against ethanol-induced gastric damage in mice. Mice were pretreated with saline (10 mL/kg, p.o.) - vehicle group, L-NAME (20 mg/kg, i.p.), ODQ (10 mg/kg i.p.), glibenclamide - GLIB (10 mg/kg i.p.), indomethacin - INDO (10 mg/kg s.c.), or ruthenium red (3 mg/kg, s.c.) before administration of McLTP$_1$ (8 mg/kg, p.o.). After 60 min, ethanol was administered (0.1 mL/10 g b.w, p.o.). Data are presented as mean ± SD. [a]$p < 0.05$ compared to vehicle and [b]$p < 0.05$ compared to McLTP$_1$ group (one-way ANOVA followed by Tukey's test; n = 8).
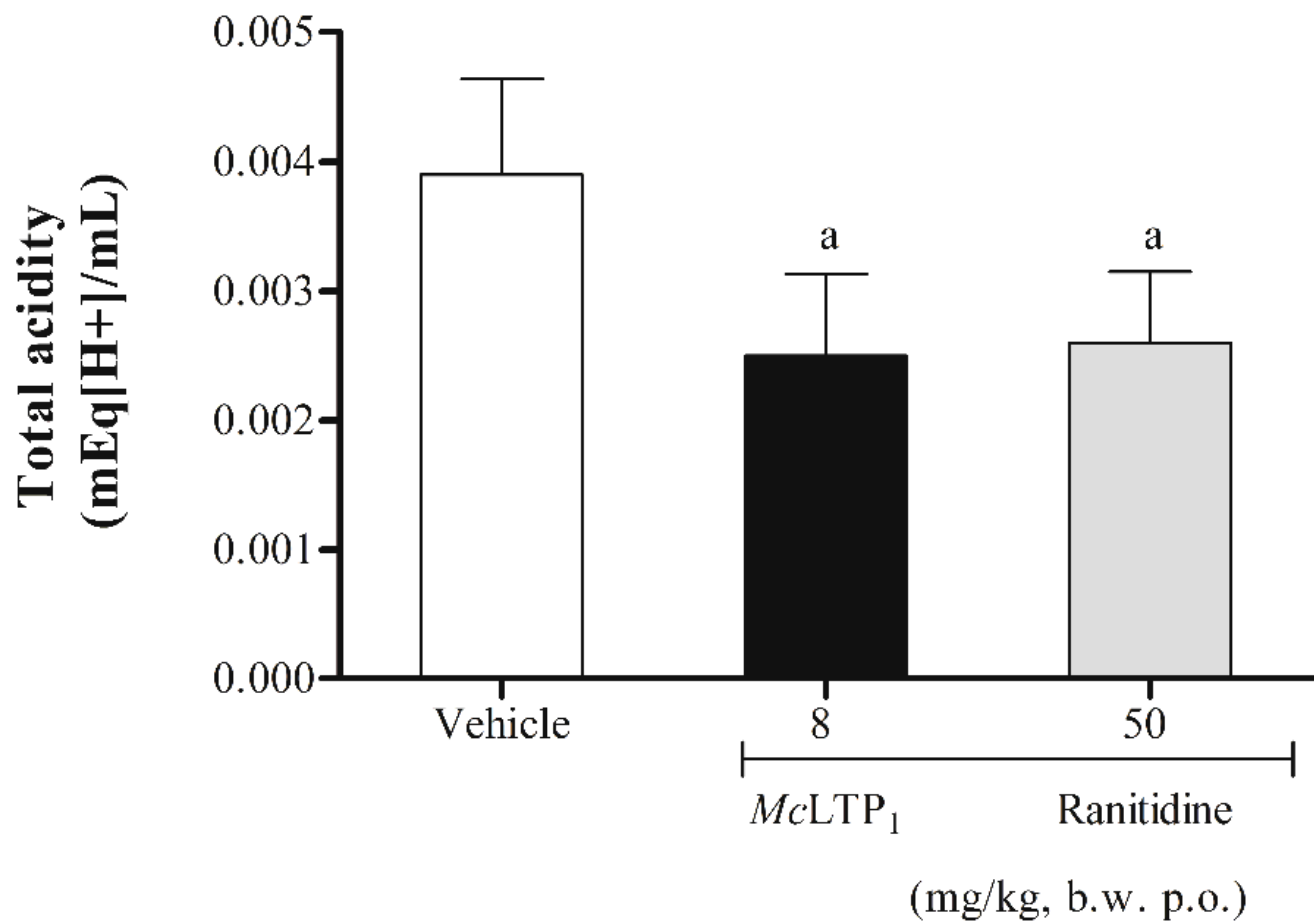
Figure 6

Effect of *Mc*LTP$_1$ on gastric acid secretion in mice submitted to the pylorus ligation. Mice were pretreated orally with vehicle NaCl 0.15 M (10 mL/kg), ranitidine (50 mg/kg), or *Mc*LTP$_1$ (8 mg/kg) 1h before the pylorus ligation procedure. Results are expressed as mean ± SD. [a]$p < 0.05$ compared with the vehicle group (one-way ANOVA followed by Tukey's test; n = 8).

Supplementary Files

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