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 ulcers. However, most of the published work 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 McLTP1, 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 McLTP1 at different doses (4, 8, or 16 mg/kg) reduced ethanol-induced gastric lesions (p < 0.05) by 40%, 84%, and 88%, respectively. In ethanol-induced gastric lesions, alterations in the levels of glutathione (GSH) (↑100%; p < 0.05) and a reduction of 45% (p < 0.05) in the levels of malondialdehyde (MDA) were demonstrated after McLTP1 administration (8 mg/kg). McLTP1 showed an anti-inflammatory effect through modulation of the cytokines IL-10 (↑33%) and TNF-α (↓54%) and was able to reduce myeloperoxidase (MPO) levels (↓95%) in gastric tissue. In addition, the gastroprotective effect of McLTP1 also involves the production of nitric oxide. The present findings reveal that McLTP1 has a gastroprotective effect dependent, at least in part, on its anti-inflammatory and antioxidant effects.

Keywords Gastric lesion · Lipid transfer proteins · Noni · Protein therapeutics · Oxidative stress · Inflammation

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 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 lesions, infiltration of neutrophils, and the release of proinflammatory 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 the gastroprotective activity of secondary plant metabolites, 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 antibiotics. Thus, peptides have emerged as a significant therapeutic class, with many natural and synthetic analogs undergoing clinical trials (Lau and Dunn 2018).

Our research group recently isolated a peptide from noni (Morinda citrifolia L.; Rubiaceae) seeds (McLTP1; UniProtKB accession number: C0HJH5) with a diverse range of pharmacological activities when orally administered to mice (Campos et al. 2016). McLTP1 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; Campos et al. 2017; Souza et al. 2018). The anti-inflammatory action mechanism of McLTP1 is related to cytokine modulation, such as TNF-α, which is involved in the pathophysiology of ethanol-induced gastric lesions (Campos et al. 2016, 2017). Hence, we hypothesized that McLTP1 could also be explored to treat gastric lesions.

This work aimed to evaluate the gastroprotective effects of McLTP1 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.

**Materials and Methods**

**Drugs and Reagents**

Tris (hydroxymethyl) aminomethane, trichloroacetic acid (TCA), thiobarbituric acid, ethanol, indomethacin, N-o-nitro-L-arginine methyl ester hydrochloride (L-NAME), ruthenium red, N-acetylcysteine (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.

**Purification of McLTP1**

McLTP1 was purified from defatted noni (M. citrifolia L.; Rubiaceae) seed flour according to the experimental procedure reported by Campos et al. (2016). The proteins were extracted from defatted noni seeds (0.05 M Tris–HCl/0.25 M NaCl, pH 8.5, 4 °C), and the crude extract was fractioned with trichloroacetic acid (TCA) to a final concentration of 2.5% (w/v) at 30 min on ice. Then, the soluble fraction was centrifuged (10,000×g for 30 min at 4 °C), and the supernatant was dialyzed, lyophilized, applied to a Sephadex G-50 column, and monitored at 280 nm.

After isolation, the purity of McLTP1 was verified by denaturing sodium dodecyl sulfate–polyacrylamide gel electrophoresis (15% SDS–PAGE) under nonreducing 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 a standard.

**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 McLTP1, 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. N° 109/16).

**Gastroprotective Activity of McLTP1**

**Effects of McLTP1 Against Ethanol-Induced Gastric Lesions in Mice**

Mice were treated orally via gavage (per os, p.o.) with either McLTP1 (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 McLTP1. 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 (mm²)/total area of stomach mucosa (mm²) × 100 (Alvarez-Suarez et al. 2011).

**Histopathological Analysis of the Stomach**

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 h. 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 and 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).

**Measurement of Malondialdehyde (MDA) and Glutathione (GSH) Levels in Stomach Tissue**

For MDA analysis, stomach samples from the different groups (naïve mice; vehicle—NaCl 0.15 M; McLTP₁ 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 µmol MDA/g of tissue (Mihara and Uchiyama 1978).

The 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 and 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/g of tissue (Sedlak and Lindsay 1968).

**Determination of Myeloperoxidase (MPO) Enzyme Activity**

Neutrophil migration on ethanol-induced gastric lesions was 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; McLTP₁ 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 ground 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) was added to the wells. After 5 min, the absorbance of the samples was measured in a microplate reader (450 nm), and the enzymatic activity of MPO was expressed as units of MPO/mg of tissue.

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

The cytokines IL-1β, TNF-α, and IL-10 were measured in the 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; McLTP₁ 8 mg/kg p.o.) were homogenized in a tissue homogenizer in phosphate-buffered PBS (100 mg tissue/1 mL buffer) and centrifuged (3000 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 (PBS with 1% BSA, 0.1% Tween 20). 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).

**Participation of Nitric Oxide (NO), Soluble Guanylate Cyclase (sGC), Potassium Channel ATP-Sensitive (KATP), Prostaglandins (PGs), and Capsaicin-Sensitive Afferent Neurons in the Gastroprotective Effect of McLTP₁ 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 pretreatment with ODQ, glibenclamide, or indomethacin and 30 min after administering L-NAME or ruthenium red (De Alencar et al. 2015 with modifications). Gastric damage induction and lesion area measurements were performed as described in Sect. 2.4.1.
Effect of McLTP₁ on Gastric Secretion

Mice were pretreated orally with vehicle NaCl 0.15 M (10 mL/kg), ranitidine (50 mg/kg), or McLTP₁ (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, pyloric ligation was performed. 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 for 20 min at 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 to be changes in gastric secretion (Shay 1945).

Statistical Analysis

The Shapiro–Wilk test was performed to determine normality. The data with p > 0.05 (Shapiro–Wilk normality test) were considered normal and analyzed using one-way analysis of variance (ANOVA), followed by Tukey’s test for multiple comparisons. The nonparametric Kruskal–Wallis comparisons test analyzed nonnormal 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 that the values show a significant difference (p < 0.05) among the compared groups.

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. № 109/16).

Results

Gastroprotective Effect of McLTP₁ on Ethanol-Induced Gastric Lesions

Ethanol administration induced intense macroscopic gastric damage in mice (14.57 ± 3.35 of lesion index). The oral treatment with McLTP₁ (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, and 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. 2A–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 McLTP₁ (8 mg/kg; p.o.) displayed marked gastric mucosal protection (p < 0.05), as evidenced by mild submucosal edema 1 (0–2), epithelial cell loss 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 McLTP₁-treated group.

Effect of McLTP₁ on Oxidative Stress and Inflammation

Compared to the basal values seen in naïve untreated mice (MDA: 22.49 ± 0.64 µmol/g tissue and GSH: 180.5 ± 4.5 µg/g tissue), ethanol significantly increased MDA gastric tissue levels by 68% (70.69 ± 12.5 µmol/g tissue) and depleted GSH levels by 41% (105.8 ± 13.8 µg/g tissue). However, pretreatment with McLTP₁ (8 mg/kg, p.o.) decreased MDA levels by 45% and increased GSH levels by 30% (229.5 ± 15.2 µg/g tissue). The results are presented in Fig. 2H and I.

Fig. 1 Gastroprotective effect of McLTP₁ on ethanol-induced gastric lesions. Mice were treated orally (p.o.) with either McLTP₁ (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 the 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)
levels by 100% compared to the vehicle \((p < 0.05)\), similar to the results observed for the treatment with NAC \((300 \text{ mg/kg; p.o.}) \) \((p > 0.05)\) (Table 1).

Pretreatment of mice with McLTP\(_1\) \((8 \text{ mg/kg; p.o.})\) significantly reduced \((p < 0.05)\) the MPO activity compared with the vehicle group (Fig. 3). Pretreatment of mice with McLTP\(_1\) \((8 \text{ mg/kg; p.o.})\) also caused a significant decrease in TNF-\(\alpha\) levels by 54\% and promoted an increase of 33\% in IL-10 levels \((p < 0.05)\) when compared to the control group. In contrast, McLTP\(_1\) was unable to significantly reduce the levels of IL-1\(\beta\) (Fig. 4).
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.) before administration of McLTP$_1$ (8 mg/kg; p.o.) did not reverse the effects 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 the mean±SD. *$p < 0.05$ compared to vehicle and $^{b}p < 0.05$ compared to McLTP$_1$ group (one-way ANOVA followed by Tukey’s test; n = 8).

After pyloric ligation, the 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 from that observed for the treatment of mice with McLTP$_1$ ($p > 0.05$) (Fig. 6).
McChloric acid (Zatorski et al. 2018). Treatment of mice with lesions, and exposes the epithelium to pepsin and hydro-
Ethanol causes high production of ROS, produces necrotic
to mice to provide the first report for future clinical use.
no significant differences in the effects observed between
we demonstrate for the first time that a protein isolated
Diverse pharmacological activities have been reported
activities. Although over 200 different compounds have
were identified in noni (Assi et al. 2017), research on pro-
tive sources for developing new drugs for treating chronic
diseases of the gastrointestinal tract because of their high
therapeutic potential and few adverse effects (Singh et al.
was not different from that presented by the positive con-
trol NAC. Considering that one of the most common side
effects of the continuous use of anti-inflammatory drugs is
the appearance of gastrointestinal injuries, the gastropro-
tective effects described by McLTP1 in this study reinforce
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 polysac-
charides have been described as potentially gastroprotec-
tive molecules from plants (Diniz et al. 2015; Neto et al.
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, and reduced toxicity and can be designed to act on
vast mechanisms, increasing the possibilities of their applica-
tion 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, McLTP1 is stable
to the main enzymes of the gastrointestinal tract (trypsin,
chymotrypsin, and pepsin) (Campos et al. 2016), which
enables the application of McLTP1 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, gastro-
intestinal cancers, and inflammatory bowel disease (Bhatt-
tacharya 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 bal-
ance, promotes lipid peroxidation and mucosal blood
e extravasation, and increases the infiltration of activated neu-
trophils (Halliwell 1994; Asmari et al. 2016). The observed
prevention of GSH depletion, combined with L-NAME
partial reversion of the gastroprotective effect of McLTP1,
suggests a potential McLTP1 action mechanism to reduce
oxidative stress.
Corroborating the data presented by Campos et al. (2017), McLTP1 showed a significant reduction in MPO
activity, an indirect parameter of neutrophil accumulation
in gastric mucosal tissues. Neutrophil release in the inflam-
matory response leads to increased gastric expression of
NF-κB, which stimulates the synthesis of proinflammatory
cytokines, including tumor necrosis factor-α (Yoo et al.
In contrast, the anti-inflammatory cytokine IL-10 is considered a potent molecule for eliminating inflamma-
tory processes involved in maintaining the homeostasis of

**Discussion**

Numerous recent studies have considered plants as alterna-
tive sources for developing new drugs for treating chronic
diseases of the gastrointestinal tract because of their high
therapeutic potential and few adverse effects (Singh et al.
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).

Fig. 6 Effect of McLTP1 on gastric acid secretion in mice subjected
to pyloric ligation. Mice were pretreated orally with vehicle NaCl
0.15 M (10 mL/kg), ranitidine (50 mg/kg), or McLTP1 (8 mg/kg) 1 h
before the pylorus ligation procedure. The results are expressed as
the mean ± SD. *p* < 0.05 compared with the vehicle group (one-way
ANOVA followed by Tukey’s test; n = 8)
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 reinforce the idea that McLTP1 may exert anti-inflammatory and antioxidant therapeutic effects via the involvement of cytokines, such as TNF-α and IL-10. In contrast, McLTP1 could not act over IL-1β, suggesting McLTP1 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 promote lipid peroxidation (Raish et al. 2018). Given that McLTP1 did not show any direct antioxidant activity, it can be reasoned that the antioxidant defenses elevated by McLTP1 described in this study are partially or entirely due to reducing these inflammatory factors.

It is 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 reversed the action of McLTP1, suggesting that the gastroprotective activity of this protein may involve the modulation of NO pathways, leading to an increase in NO levels in the gastric mucosa. Our results also showed that McLTP1 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 McLTP1. These results indicate that sGC, KATP, PGs, and capsaicin-sensitive sensory receptors are not involved in the effects of McLTP1. Our findings are similar to those described by Pinheiro et al. (2013). Proteins isolated from the latex of Himatanthus drasticus showed gastroprotective effects 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 McLTP1 as a strong candidate for developing a new drug of protein origin to treat gastrointestinal tract disorders.

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 McLTP1 are of significant clinical importance. However, standardized pharmacological safety studies will be necessary to ensure safe use.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10989-022-10440-y.

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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 MHLP contributed to financial obtaining and support. The authors declare that they have no conflicts of interest.

Declarations

Competing interests The authors declare no competing interests.

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