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Article

Gastroprotective Effect of oenothein B: a Romising Macrocyclic Ellagitannin

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ABSTRACT

A peptic ulcer disease is a significant gastrointestinal disorder with a high prevalence of 80% in developing countries and 40% in developed countries. Oenothein B, a macrocyclic ellagitannin, has emerged as a potential therapeutic agent for this condition. The aim of this study was to investigate the anti-ulcer activity of Oenothein B and elucidate the underlying mechanisms involved in its gastroprotective effects. The anti-ulcer activity of Oenothein B was assessed using various experimental models, including ulcers induced by indomethacin, HCl/ethanol, and pyloric ligation. Several parameters were evaluated, including the quantification of volume, pH, total gastric acidity secretion, as well as catalase and superoxide dismutase activity. The role of prostaglandins in mediating the effects of Oenothein B was also examined. The results demonstrated that Oenothein B exhibited significant anti-ulcer activity when administered intraduodenally at a dose of 15 mg/kg in an acute protocol of induced ulcers. This treatment resulted in a reduction in volume and total gastric acidity secretion. Moreover, oral administration of Oenothein B was found to increase catalase and superoxide dismutase activity in the gastric mucosa. These findings suggest that Oenothein B exerts its anti-ulcer effects by reducing gastric acid secretion and enhancing the activity of antioxidant enzymes. Additionally, the modulation of endogenous prostaglandin levels appears to play a role in mediating these effects. In conclusion, Oenothein B demonstrates promising anti-ulcer activity and may serve as a potential therapeutic option for the management of peptic ulcer disease. Further investigations are warranted to elucidate the detailed mechanisms of action and evaluate its efficacy and safety in clinical settings.
Keywords: gastric cytoprotection; mucosal protection; Oenothein B; animal models; ulcers.

RESUMO

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A úlcera péptica é um distúrbio gastrointestinal significativo com alta prevalência de 80% nos países em desenvolvimento e 40% nos países desenvolvidos. A Oenotheina B, um elagitanino macrocíclico, surgiu como um potencial agente terapêutico para esta condição. O objetivo deste estudo foi investigar a atividade antiúlcera da Oenotheina B e elucidar os mecanismos subjacentes envolvidos nos seus efeitos gastroprotetores. A atividade antiúlcera da Oenotheina B foi avaliada usando vários modelos experimentais, incluindo úlceras induzidas por indometacina, HCl/etanol e ligadura pilórica. Vários parâmetros foram avaliados, incluindo a quantificação de volume, pH e acidez total, bem como atividade de catalase e superóxido dismutase. O papel das prostaglandinas na mediação dos efeitos da Oenotheina B também foi examinado. Os resultados demonstraram que a Oenotheina B exibiu atividade antiúlcera significativa quando administrada intraduodenalmente numa dose de 15 mg/kg no protocolo agudo de úlceras induzidas. Este tratamento resultou em redução do volume e da secreção total da acidez gástrica. Além disso, descobriu-se que a administração oral de Oenotheina B aumenta a atividade da catalase e da superóxido dismutase na mucosa gástrica. Estas descobertas sugerem que a Oenotheina B exerce os seus efeitos antiúlcera, reduzindo a secreção de ácido gástrico e aumentando a atividade de enzimas antioxidantes. Além disso, a modulação dos níveis endógenos de prostaglandinas parece desempenhar um papel na mediação destes efeitos. Em conclusão, a Oenotheina B demonstra atividade antiúlcera promissora e pode servir como uma opção terapêutica potencial para o tratamento da úlcera péptica. Mais investigações são necessárias para elucidar os mecanismos detalhados de ação e avaliar sua eficácia e segurança em ambientes clínicos.
Palavras-chave: citoproteção gástrica; proteção da mucosa; Oenotheina B; modelos animais; úlceras.

Introduction

Peptic ulcer disease (PUD) is widely recognized as a significant gastrointestinal disorder that affects a substantial portion of the global population (Amirshahrokhi & Khalili 2015). Its estimated prevalence is as high as 80% in developing countries and 40% in developed countries (Balan et al. 2015).

The mechanism of gastric mucosal cytoprotection involves a reduction in the activity of parietal cells, which regulate the secretion of hydrochloric acid, and an increase in mucus, bicarbonate, blood flow, cell regeneration, and the release of prostaglandins (PGs), as well as endogenous factors like epidermal growth factor (Mota et al., 2009; Batista et al. 2004). Despite advancements in our understanding of this disease, the treatment of PUD still primarily relies on the inhibition of gastric acid secretion, typically through the use of H2 receptor blockers (e.g., ranitidine and famotidine), anticholinergics (e.g., pirenzepine and telenzepine), or proton pump inhibitors (e.g., omeprazole and lansoprazole) (Lászlo et al. 2020). However, the prolonged use of these medications has demonstrated side effects, including nephrotoxicity, hepatotoxicity, gynecomastia, constipation, hypergastrinemia, impotence, and a risk of relapses and drug interactions (Mota et al., 2009; Martins et al. 2017; Ateufack et al. 2015).

Lipid peroxidation, mediated by reactive oxygen species (ROS), plays a role in cell membrane damage and is implicated in the pathogenesis of gastric ulcers (Zakaria et al. 2014). ROS react with cellular lipids, leading to the formation of lipid peroxides, which are further metabolized into malondialdehyde, an important product of lipid peroxidation (Zakaria et al. 2014). Therefore, antioxidants play a crucial role in defense mechanisms against ROS, and compounds with antioxidant properties may exhibit gastroprotective effects (Balan et al. 2015; Bhattacharyya et al. 2014).

Oenothein B (Fig.1) is a soluble macrocyclic ellagitannin characterized by the presence of twenty-two phenolic hydroxyl groups (Hatano et al. 1990). Among its diverse biological activities (Yoshida et al. 2018), the immunomodulatory and antioxidant properties of oenothein B are particularly noteworthy (Okuyama et al. 2021; Li et al. 2020).

In a previous study, our research group demonstrated the gastroprotective effect of the hydroacetonic fraction of *Eugenia uniflora* L. leaf extract in various models of gastric lesions (Martins et al. 2017). This effect was attributed to the presence of numerous phenolic compounds, including oenothein B (De-Faria et al. 2012). Given the previously observed effects of oenothein B as a phytoconstituent isolated from this plant, our research group embarked on an investigation to explore the anti-ulcer activity of oenothein B and the potential pathways involved in gastroprotection using different experimental protocols with rodents. This study advances

our understanding of the gastroprotective potential of oenothein B and provides new insights into its biological effects, with important implications for potential health benefits derived from this compound.

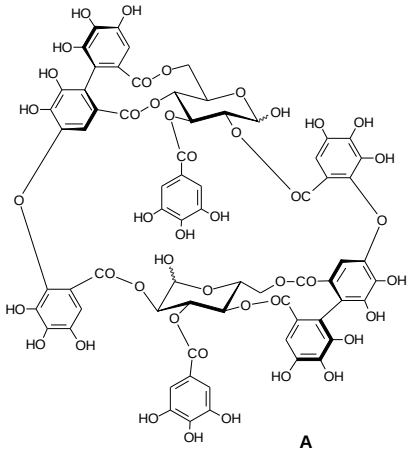

Figure 1: Chemical structure of oenothein B. (A). Source: Prepared by the authors

Materials and methods

Chemicals and drugs

The following drugs and chemicals were utilized: absolute ethanol (Quimex, São Paulo, SP, Brazil), carbenoxolone, indomethacin, and alcian blue (Sigma-Aldrich, St. Louis, MO, USA); ranitidine (Teuto, Anapólis, Brazil), Tween 80 (Sigma-Aldrich), sucrose (Labsynth, Diadema, SP, Brazil), magnesium chloride (Quimibras, Rio de Janeiro, RJ, Brazil), sodium acetate (Vetec, Duque de Caxias, RJ, Brazil), sodium hydroxide (Cristália, São Paulo, SP, Brazil), and CO_2 (White Martins, São Paulo, SP, Brazil).

Oenothein B was obtained from the Laboratory of Chemistry of Natural Products at the Federal University of Goiás, Brazil (Fortes et al. 2015), and was first solubilized in filtered water with Tween 80 (2%). Subsequently, oenothein B and drug concentrations were adjusted and administered at 10 ml/kg body weight (BW), while control groups received vehicle: filtered water with Tween 80 (2%). Indomethacin was dissolved in a 5% sodium bicarbonate solution. All drugs and reagents were prepared immediately before use. Other drugs were dissolved in distilled water.

Animals

A total of 120 adult male Swiss mice (6–8 weeks, 25-35 g) and 35 Wistar rats (6–8 weeks, 200-250 g) obtained from the colony of the Federal University of Goiás were used in this study. The animals were randomly divided into four groups: (G1) control group (5x), (G2) Ranitidine 50 mg/kg (3x), (G3) carbenoxolone 200 mg/kg (1x), (G4) low dose of Oenothein B (7.5 mg/kg) (1x), (G5) moderate dose of Oenothein B (15 mg/kg) (3x), (G6) high dose of Oenothein B (30 mg/kg) (2x), (G7) Saline 10 ml/kg + Vehicle 10 ml/kg, (G8) Saline 10 ml/kg + Oenothein B 15 mg/kg, (G9) Saline 10 ml/kg + Carbenoxolone 200 mg/kg, (G10) Indomethacin 10 mg/kg + Vehicle 10 ml/kg, (G11) Indomethacin 10 mg/kg + Oenothein B 15 mg/kg, (G12) Indomethacin 10 mg/kg + Carbenoxolone 200 mg/kg, (G13) Vehicle + Ethanol, (G14) Carbenoxolone 200 mg/kg + Ethanol, (G15) Oenothein B 15 mg/kg + Ethanol, (G16) Sham, (G17) Saline + Vehicle, (G18) Saline + Oenothein B 15 mg/kg, (G19) Indomethacin 30 mg/kg + Vehicle, (G20) Indomethacin 30 mg/kg + Oenothein B 15 mg/kg. The animals were housed in controlled temperature (22-23 ºC) and subjected to a 12-

hour light/dark cycle with ad libitum access to water and food. All experiments were conducted in accordance with ethical protocols for animal research, performed between 8:00 a.m. and 4:00 p.m., and were approved by the institutional committee for animal experimentation (Protocol CEUA/UFG 038/14). In all protocols, the animals were euthanized in a CO_2 chamber.

Indomethacin-induced gastric lesions in gastric mucosa of mice

Indomethacin-induced gastric lesions were created following the adapted method from (Djahanguiri 1969). For this, after fasting for 16 h, the animals (n=8) received vehicle (distilled water, 10 ml/kg), oenothein B (7.5, 15, or 30 mg/kg) or ranitidine (50 mg/kg BW), through oral pathway and after 60 min of this treatment, all animals were submitted to a After a 16-hour fast, the animals (n=8) received vehicle (distilled water, 10 ml/kg), oenothein B (7.5, 15, or 30 mg/kg), or ranitidine (50 mg/kg BW) orally. After 60 minutes of this treatment, all animals were subcutaneously administered indomethacin (30 mg/kg), an inhibitor of the cyclooxygenase (Cox) enzyme, for gastric ulcer induction. The animals were euthanized 6 hours after indomethacin administration, and their stomachs were removed to evaluate ulcer lesions using the Lesion Index (LI) for estimating the percentage of inhibition. For LI determination, the stomachs were dissected along the greater curvature, and the inner surface was examined with a magnifying glass to evaluate lesions (Rios et al. 2010; Szabo et al. 1985).

HCl/Ethanol-induced gastric ulcer model

HCl/Ethanol-induced gastric lesions were created following the adapted method from (Mizui & Doteuchi 1983). After a 16-hour fast, the animals received vehicle (distilled water, 10 ml/kg), oenothein B (15 mg/kg BW), or carbenoxolone (200 mg/kg BW) orally. After 60 minutes of this treatment, all animals received oral administration of 0.45 M HCl/Ethanol 60% (10 ml/kg) for gastric ulcer induction. The animals were euthanized 1 hour after the HCl/Ethanol administration, and their stomachs were dissected along the greater curvature, photographed, and the area of lesions was measured using AutoCAD software® v.21. The area of the gastric mucosal lesions in each animal was calculated and expressed as a percentage relative to the total area of the stomach using the formula: % inhibition = (UI control − UI treated) / UI control × 100.

Gastric lesions induced by pylorus ligation in gastric mucosa of mice

Gastric lesions were induced by pylorus ligation according to the adapted method from (Dai & Ogle 1973). After a 16-hour fast, the animals (n=8) received vehicle (distilled water, 10 ml/kg), oenothein B (15 mg/kg BW), or ranitidine (50 mg/kg BW) intraduodenally. Four hours after this treatment, all animals were euthanized; their stomachs were dissected along the greater curvature, and the LI was determined for ulcer lesions evaluation. Gastric content was also collected, and the volume, free acidity (pH), and total acidity were quantified.

***Ex vivo* biochemical assays in gastric mucosa of mice**

For biochemical assays, after a 16-hour fast, the animals (n=8) received vehicle (distilled water, 10 ml/kg), oenothein B (15 mg/kg BW), or carbenoxolone (200 mg/kg BW) orally. One hour after this treatment, the harmful agent (80% Ethanol, 10 ml/kg) was orally administered. After 1 hour, all animals were euthanized, and their stomachs were dissected along the greater curvature. The mucosa was scraped until completely removed and homogenized in 20% phosphate buffer (50 mM, pH 7.0). The homogenate was centrifuged at 4000 rpm for 20 minutes at 2 °C. The supernatant was assessed for protein content according to and used to assess catalase (CAT) or total superoxide dismutase (SOD) activity (Bradford 1976).

Catalase activity

Catalase enzyme activity was measured based on the decomposition of H_2O_2 consumption or liberation of O_2. The rate of decomposition of H_2O_2 in the stomachs of mice after gastric lesions induced with 80% ethanol was spectrophotometrically measured from changes in absorbance at 240 nm. The CAT activity was expressed as pmol/mg of protein (Boveris & Chance 1973).

Superoxide dismutase (SOD) activity

Superoxide dismutase activity was measured based on the ability of the SOD enzyme to inhibit the autoxidation of epinephrine. The supernatant of stomachs from mice after gastric lesions induced with 80% ethanol was incubated with epinephrine bitartrate (60 mM), and the sample color intensity was measured at 480 nm (Misra & Fridovich 1974).

Determination of prostaglandin E2 synthesis in gastric mucosa of rats

After a 16-hour fast, the rats (n=7) received saline (NaCl 0.9%, 10 ml/kg) or indomethacin (30 mg/kg) subcutaneously. One hour after this pre-treatment, the animals received an oral dose of vehicle, oenothein B (15 mg/kg), or were in the sham group. After 1 hour, all animals were euthanized, the stomachs were dissected, and the mucosa was scraped, weighed, and suspended in 1 ml of sodium phosphate buffer (10 mM, pH 7.4). The remaining solution was incubated in a water bath (Dubnoff Tecnal - Brazil) at 37 °C for 20 minutes. PGs were determined in the present buffer content using the Biotrak TM® Prostaglandin E2 direct assay kit (RPN 222 - Amesham Bioscience) (Curtis et al. 1995).

Statistical analysis

Data were expressed as mean ± standard error of the mean (SEM) and subjected to the Student's t-test for two comparisons of independent samples or analyzed using analysis of variance with Tukey-Kramer's test when two or more groups were examined (Drummond & Tom 2011). Statistical significance was considered when $p < 0.05$ and was processed using GraphPad Prism software (GraphPad version 6.00, San Diego, CA).

Results

Effect of oenothein B in indomethacin-induced ulcers in mice

Treatment with Oenothein B (7.5, 15, or 30 mg/kg) exhibited a significant and dose-dependent reduction in the Lesion Index (LI) in gastric mucosa lesions induced by indomethacin administration (Table 1). The highest dose of Oenothein B (30 mg/kg) demonstrated the most substantial reduction in LI, underlining its efficacy in countering indomethacin-induced ulcers. It's noteworthy that the positive control group treated with ranitidine (50 mg/kg) also exhibited a significant reduction in LI, reinforcing the potential therapeutic value of Oenothein B in this context.

Table 1. Effect of oral treatment of oenothein B or ranitidine in indomethacin-induced gastric lesions in mice.

Treatments (p.o.)	Dose (mg/kg)	Lesions index	Reduction (%)
Vehicle	-	10.0 ± 0.8	-
Ranitidine	50	6.0 ± 0.5**	40*0
Oenothein B	7.5	7.2± 0.6*	28.0
	15	5.5 ± 0.6**	45.0
	30	5.2 ± 0.4**	48*0

Results are expressed as mean ± SEM of the LI of each group (n = 8). One-way ANOVA followed by the Tukey post-hoc test. * p ≤ 0.05; ** p ≤ 0.01 compared with the vehicle group. Source: Prepared by the authors (2023).

Effect of oenothein B in HCl/Ethanol-induced ulcer

The administration of HCl/Ethanol solution resulted in extensive lesions in the gastric mucosa of the control animals. These lesions were characterized by numerous red or dark brown hemorrhagic areas of varying sizes along the stomach, resulting in an ulcerated area of 55.6 ± 4.75%. In contrast, the group treated with carbenoxolone (200 mg/kg) showed a remarkable reduction in the ulcerated area to 19.2 ± 2.1%, representing a significant 65% of gastroprotection. Treatment with Oenothein B (15 mg/kg) also led to a substantial 42.5% reduction in the ulcerous area compared to the control group, highlighting its gastroprotective potential. For a visual representation of these effects, please refer to Fig. 2, and for detailed percentages of the ulcerated area (Table 2).

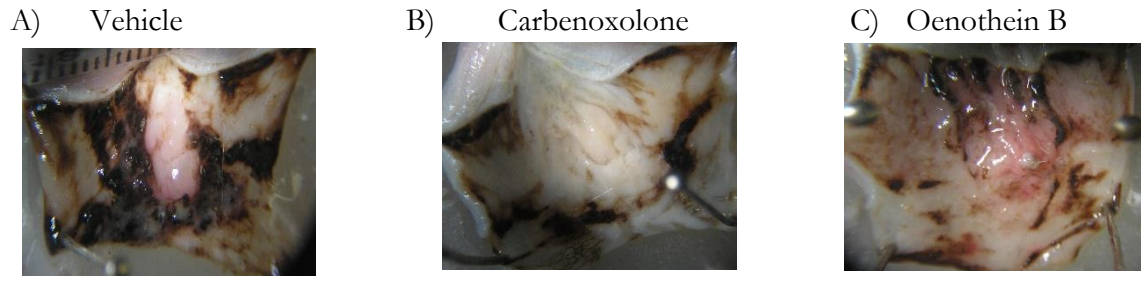

Figure 2. Gastroprotective effect of oenothein B or carbenoxolone on the HCl/Ethanol induced gastric lesions in mice. Source: Prepared by the authors.

Table 2. Effect of oral treatment of the oenothein B or carbenoxolone on the HCl/Ethanol induced gastric lesions model in mice.

Treatments (p.o.)	Dose (mg/kg)	Ulcerated area (%)	Reduction (%)
Vehicle	-	55.6 ± 4.7	-
Carbenoxolone	200	19.2 ± 2.1***	65.5
oenothein B	15	32.0± 3.1***	42.5

Results are expressed as mean ± SEM of the ulcerated area (%) of each group (n = 8). One-way ANOVA followed by the Tukey post-hoc test. *** p ≤ 0.001 compared with the vehicle group. Source: Prepared by the authors (2023).

Effect of oenothein B in gastric lesions induced by pylorus ligation

In the model of gastric ulcers induced by pyloric ligation, treatment with Oenothein B (15 mg/kg) significantly reduced the Lesion Index (LI), further validating its gastroprotective effects (Table 3). Similar to the previous results, the positive control group treated with ranitidine (50 mg/kg) also demonstrated a reduction in LI.

Table 3. Effect of intraduodenal treatment of the oenothein B or ranitidine in the sample of induced gastric lesions in pyloric ligation in mice.

Treatments (p.o.)	Dose (mg/kg)	Lesions index (IL)	Reduction (%)
Vehicle	-	10.10 ± 0.88	-
Ranitidine	50	6.62 ± 0.37**	34.5
Oenothein B	15	6.25± 0.49**	38.1

Results are expressed as mean ± SEM of the LI of each group (n = 8). One-way ANOVA followed by the Tukey test as a post-test. *** p ≤ 0.001 compared with the vehicle group. Source: Prepared by the authors (2023).

Effect of oenothein B in parameters involved in gastric acid

Oenothein B (15 mg/kg) treatment effectively reduced the volume of gastric juice and total acidity, indicating its ability to modulate gastric acid secretion (Table 4). This reduction in gastric acid secretion aligns with the gastroprotective properties of Oenothein B. Additionally, the positive control ranitidine (50 mg/kg) also demonstrated a reduction in the volume of gastric juice and total acidity, while simultaneously increasing the pH of gastric acid secretion, further emphasizing its effectiveness in this regard.

Table 4. Effect of treatment via intraduodenal with oenothein B or ranitidine in parameters of gastric juice in mice subjected to pyloric ligation.

Treatments (i.d)	Dose (mg/kg)	Volume (ml)	pH	Total acid (mEq[H^+] /L/4h)
Vehicle	-	2.60 ± 0.09	3.27 ± 0.3	5.93 ± 0.5
Ranitidine	50	2.32 ± 0.03**	5.11 ± 0.1***	2.45 ± 0.3***
Oenothein B	30	2.25 ± 0.06**	3.31 ± 0.3	3.21 ± 0.5**

Results are expressed as mean ± SEM of each group (n = 8). One-way ANOVA followed by the Tukey test as a post-test. ** p ≤ 0.01*** p ≤ 0.001 compared with the vehicle group. Source: Prepared by the authors (2023).

Effect of oenothein B on HCl/ethanol-induced gastric mucosal lesion

The HCl/ethanol protocol induced a substantial gastric lesion area of 55.7 ± 2.6%. Oenothein B (15 mg/kg) demonstrated its capacity to reduce the ulcerated area to 38.2 ± 3.5%, emphasizing its protective effects against HCl/ethanol-induced gastric mucosal lesions. Notably, pretreatment with indomethacin reversed the gastroprotective activity of Oenothein B, resulting in an increased ulcerated area of 48.1 ± 3.5% (Table 5).

Table 5. Role of PGs in the gastro protective effect of oenothein B against HCl/ethanol-induced gastric injury in mice.

Pretreatment (s.c.)	Treatments (p.o.)	Ulcerated area (%)
Saline 10 ml/kg	Vehicle 10 ml/kg	55.7 ± 2.6
Saline 10 ml/kg	Oenothein B 15 mg/kg	38.2 ± 3.5***
Saline 10 ml/kg	Carbenoxolone 200 mg/kg	31.9 ± 3.9***
Indomethacin 10 mg/kg	Vehicle 10 ml/kg	58.5 ± 3.9
Indomethacin 10 mg/kg	Oenothein B 15 mg/kg	48.1 ± 3.5$^{\#}$
Indomethacin 10 mg/kg	Carbenoxolone 200 mg/kg	53.0 ± 3.0$^{\#\#\#}$

Animal groups were pretreated with saline or indomethacin 60 min before oral treatment with vehicle, oenothein B, or carbenoxolone. Results are expressed as mean ± SEM of the ulcerated area (%) of each group (n = 8). One-way ANOVA followed by the Tukey test as a post-test. (n) Number of animals in each group. *** $p \leq 0.001$ indicates significant difference of the saline + vehicle group vs saline + oenothein B or saline + carbenoxolone or $^{\#}p \leq 0.05$, $^{\#\#\#}p \leq 0.001$ indicates representative difference of the indomethacin + vehicle group vs indomethacin + oenothein B or indomethacin + carbenoxolone. Source: Prepared by the authors (2023).

Effect of oenothein B in catalase activity

Pretreatment with Oenothein B (15 mg/kg) or carbenoxolone (200 mg/kg) notably increased catalase (CAT) activity by 20.3% and 61.5%, respectively, compared to the control group (Table 6). These findings highlight the antioxidant properties of Oenothein B and carbenoxolone. Conversely, treatment with 80% ethanol in the control group resulted in a 17% reduction in CAT activity compared to the sham group, which had a concentration of 484 ± 16.7 nmol/mg of protein.

Effect of oenothein B in superoxide dismutase (SOD) activity

The activity of SOD in the 80% ethanol-treated control group showed a significant reduction compared to the sham group. This reduction was effectively reversed with pretreatment with Oenothein B (15 mg/kg) or carbenoxolone (200 mg/kg), demonstrating a significant antioxidant effect represented by SOD activity (Table 6). These results emphasize the potential of Oenothein B and carbenoxolone in counteracting oxidative stress.

Table 6. Effect of oral treatment with oenothein B or carbenoxolone in CAT or SOD activity after Ethanol-induced gastric lesions.

Treatment (p.o.)	Dose (mg/kg)	Catalase (nmol/mg of protein)	SOD (U/mg of protein)
-	-	484 ± 16.7	9.74 ± 0.34
Vehicle + Ethanol	-	387 ± 14.7*	7.95 ± 0.44**
Carbenoxolone + Ethanol	200	654 ± 31,6$^{\#\#}$	11.21 ± 0.35$^{\#\#\#}$
Oenothein B + Ethanol	15	485 ± 22.4$^{\#}$	9.42 ± 0.29$^{\#}$

ılts are expressed as mean ± SEM of each group (n = 8). One-way ANOVA followed by the Tukey test as a post-test. *$p \leq 0.05$ or **$p \leq 0.01$ in comparison to the sham group; 0.05, $^{\#\#}p \leq 0.01$ or $^{\#\#\#}p \leq 0.001$ in comparison to control group. Source: Prepared by the authors (2023).

Effect of oenothein B on PGE_2 synthesis

Indomethacin induced a depletion of prostaglandin E_2 (PGE_2) in the group treated with the vehicle (Fig. 3). Oenothein B was able to maintain baseline levels of PGE_2, similar to the sham group and the vehicle group. However, when co-administered with indomethacin, Oenothein B did not maintain PGE_2 levels, suggesting a potential interaction between Oenothein B and indomethacin in the regulation of PGE_2 synthesis.

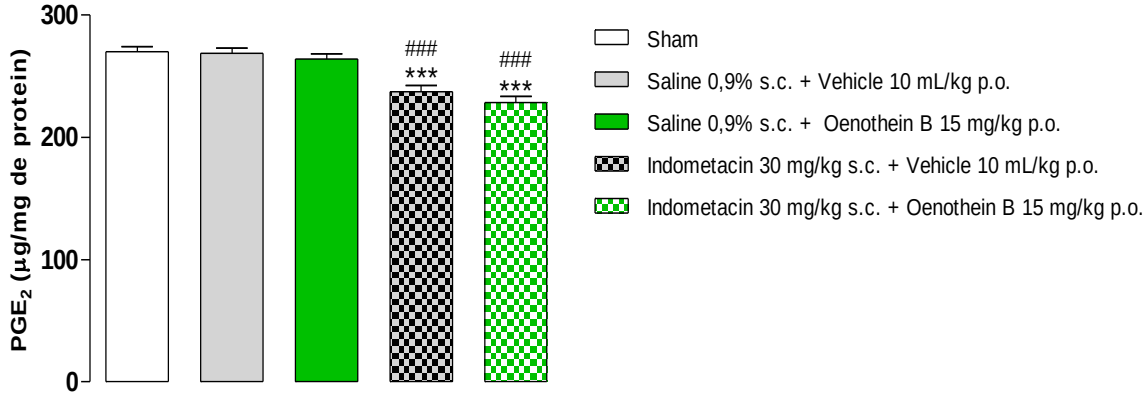

Figure 3. Effect of oenothein B on the synthesis of prostaglandin E_2 (PGE_2) in rats. Source: Prepared by the authors.

Results are expressed as mean ± SEM of the ulcerated area (%) of each group (n = 7). One-way ANOVA followed by the post hoc Tukey test. (n) Number of animals in each group. *** $p \leq 0.001$ indicates significant difference of the sham group or ### = $p \leq 0.001$ and of the group pretreated with saline + oenothein B. Source: Prepared by the authors (2023).

Discussion

The functional integrity of the gastric mucosa depends on a delicate balance between aggressive and protective factors. Successful pharmacological treatments for gastroprotection rely on the reduction of acidic gastric secretion and the enhancement of cytoprotective mechanisms in the gastric mucosa (Caldas et al. 2014). This study provides insights into the potential gastroprotective properties of Oenothein B and the underlying mechanisms responsible for its efficacy.

Indomethacin-induced gastric ulcers stem from the inhibition of cyclooxygenase (Cox), a pivotal mechanism in the pathogenesis of gastric injury induced by non-steroidal anti-inflammatory drugs (Falcão et al. 2013). Cox inhibition leads to reduced prostaglandin levels (PGs) in the gastric mucosa, resulting in decreased mucus and bicarbonate secretion, blood flow reduction, and leukocyte accumulation (Wallace 2001). In this study, Oenothein B effectively inhibited the formation of gastric lesions induced by indomethacin, implying that PGs may be involved in the gastroprotective activity exhibited by this ellagitannin.

Gastric ulcers induced by ethanol have multifactorial origins, with ethanol causing lesions directly through local injury or indirectly by increasing the production of reactive oxygen species (ROS) (Rozza et al. 2014). Ethanol leads to the reduction of gastric mucosal protective factors, such as the mucus barrier and non-protein sulfhydryl groups, particularly glutathione (GSH). Moreover, the combination of HCl/Ethanol contributes to stasis of gastric blood flow, exacerbating hemorrhagic necrosis and tissue injury (Konturek et al. 1998). The results indicate that Oenothein B significantly reduced the ulcerated area by 42.5%, suggesting potential

cytoprotective activity. Ethanol acts directly on gastric mucosal cells, causing lesions by reducing blood flow and mucus production. Agents that enhance mucosal defensive factors, including mucus, antioxidant enzymes, and PGs, can inhibit ethanol-induced ulcers (Caldas et al. 2014).

Reactive oxygen species (ROS) play a pivotal role in cell homeostasis and are implicated in the pathogenesis of ethanol-induced gastric ulcers (Sidahmed et al. 2015). Ethanol-induced oxidative stress increases ROS formation while diminishing cellular antioxidant defenses, leading to lipid peroxidation and protein oxidation (Rocha et al. 2011). Oenothein B effectively increased catalase (CAT) activity, re-establishing levels similar to the sham group, thereby counteracting the destructive effects of oxidative damage. Several studies have demonstrated Oenothein B's various biological activities, including antioxidant and immunomodulatory properties (Yoshida et al. 2018). Its antioxidant capacity has been established through various methods, such as the DPPH and OH radicals tests (Li et al. 2020). Epilobium species that have a high proportion of macrocyclic tannins, such as oenothein B, showed that *Epilobium parviflorum* extract was able to scavenge free radicals comparable to the antioxidants Trolox and ascorbic acid (Tóth et al. 2009).

Oenothein B has been shown to activate phagocyte functions, inducing intracellular Ca^{2+} flux, ROS production, NF-kB activation, pro-inflammatory cytokine production, and the regulation of the Keap1/Nrf2 signaling pathway, which alleviates oxidative stress. Additionally, Oenothein B induces apoptosis in a ROS-dependent manner and inhibits cell growth (Schepetkin et al. 2009; Pei et al. 2019).

Gastric acid secretion plays a crucial role in the pathogenesis of gastric ulcers induced by pylorus ligation (Muthuraman & Sood 2010). The pyloric ligation model also yields changes in biochemical parameters of gastric contents, such as volume (ml), free acidity (pH), and total acidity. Accumulation of acid and pepsin in these samples leads to auto-digestion and ulceration of the stomach (Bharti et al. 2010). Animals pretreated with Oenothein B showed a significant reduction in volume (ml) and total acidity, indicating that part of Oenothein B's gastroprotective effect may involve systemic actions, even when administered directly into the duodenum. This aligns with results obtained from the aqueous fraction of hydroacetonic leaf extract of *Eugenia uniflora* L., from which Oenothein B was derived (Martins et al. 2017).

PGE_2 regulates mucus and bicarbonate secretion, inhibits gastric acid secretion, and promotes angiogenesis and epithelial cell renewal (Matsui et al. 2011). Suppression of prostaglandin synthesis by non-steroidal anti-inflammatory drugs (NSAIDs) like indomethacin increases susceptibility to mucosal lesions and gastrointestinal ulceration (Atay et al. 2000). In our study, pretreatment with indomethacin reversed the gastroprotective effect of Oenothein B in the HCl/ethanol-induced ulcer model, suggesting that this effect may be linked to the modulation of PGs. However, Oenothein B was unable to restore normal PGE_2 levels in the gastric mucosa of animals pretreated with indomethacin, indicating a complex interplay between Oenothein B and indomethacin in regulating PGE_2 synthesis.

In summary, our findings highlight the potential gastroprotective role of Oenothein B, with a particular emphasis on its ability to modulate various factors, including oxidative stress, PGs, and gastric acid secretion. Further investigations are warranted to fully elucidate the mechanisms underlying its protective effects and its potential as a therapeutic agent for gastric ulcers. Expanding upon these findings, future studies may explore the specific pathways and signaling molecules involved in Oenothein B's actions, paving the way for potential therapeutic applications in the treatment of peptic ulcer disease.

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Conflict of interest

The authors have no competing interests to declare that are relevant to the content of this article.

Ethical Approval

The authors declare that the experiments with animals were performed in compliance with international rules on the care and use of laboratory animals. This study was approved by the Animal Ethics Committee of Federal University of Goiás - UniEVANGÉLICA (Protocol CEUA/UFG 038/14).

Data availability

Data generated or analyzed during this study are provided in full within the published article.

Author Contribution

Conceptualization: JLRM, DMS, EAC; Methodology: JLRM, DMS,TSO, FFR; Formal analysis and investigation: IFF, DMS, JOF; Writing - original draft preparation: JLRM, TSO, ESG; Writing - review and editing: EAC, ONS, EMHP; Funding acquisition: EAC, EMHP, IFF, ESG; Resources: JLRM, DMS, ALF, TSO; Supervision: ONS, EAC, ALF, SC, TSO. All authors have read and approved the fnal submission.

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