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Gastroprotective effects of essential oil from *Protium heptaphyllum* on experimental gastric ulcer models in rats

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Abstract: Peptic ulcers are a common disorder of the entire gastrointestinal tract, its etiology has not been completely elucidated. The basic physiopathological of peptic ulcers result from an imbalance between some endogenous aggressive factor and cytoprotective factors. The treatment of this disease is usually done with antacids or proton pump, but are currently being used plants derivated compounds. We evaluated the gastroprotective properties and its possible mechanisms of action of the essential oil from *Protium heptaphyllum* (Aubl.) Marchand, Burseraceae (BB). The formation of ulcers, were evaluated in three experimental models, through the induction of gastric lesions by ethanol, nonsteroidal anti-inflammatory drugs and acetic acid. The mechanisms of action were evaluated through the pylorus ligature experiment, western blot, GSH, GR, SOD, GPx, MDA and MPO activities. BB significantly inhibited the formation of ulcers induced by the three different models, increased the GSH and GR levels and maintained the same levels of SOD and GPx of the sham group, inhibited MPO and MDA, did not produce significant modification in gastric juice content and showed increased COX-2 and EGF. BB exerts its gastroprotective activity, possibly, by increasing COX-2 and EGF expression and due to its possible antioxidant property.

Article

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Introduction

Peptic ulcer is a common disorder of the entire gastrointestinal tract, they occur mainly in the stomach and the proximal duodenum (Mayty et al., 2003). Despite great advances in the understanding of the peptic ulcer illness, its etiology has not been completely elucidated. The basic physiopathological of gastric ulcer results from an imbalance between some endogenous aggressive factor(s) [hydrochloric acid, pepsin, refluxed bile, leukotrienes, reactive oxygen species (ROS)] and cytoprotective factors, which include the function of the mucus-bicarbonate barrier, surface active phospholipids, prostaglandins (PG), mucosal blood flow, cell renewal and migration, nonenzymatic and enzymatic antioxidants and some growth factors (Bandyopadhyay et al., 2001; Bhattacharjee et al., 2002).

Although recent advances in our understanding have highlighted the multi-factorial pathogenesis of peptic ulcers, secretion of gastric acid is still recognized as a central component of this disease. Therefore, the main therapeutic target is the control of this secretion using antacids, H2 receptor blockers (ranitidine, famotidine) or proton pump blockers (omeprazole and lansoprazole) (Rao et al., 2004). However, nowadays, gastric ulcer therapy faces a major drawback because most of the drugs currently available in the market show limited efficacy against gastric diseases and are often associated with severe side effects (Bandyopadhyay et al., 2002; Lehne, 1998).

In this context, the use of medicinal plants for the prevention and treatment of different pathologies is in continuous expansion worldwide (Mota et al., 2009). Natural products are gaining space and importance in the pharmaceutical industry as well as inspiring the search for new potential sources of bioactive molecules (Cechinel-Filho & Yunes, 2001; Schmeda-Hirschmann & Yesilada, 2005). The natural active compounds classes or secondary metabolites as alkaloids, flavonoids, terpenoids, tannins and others have attracted researchers to investigate their chemical, toxicological and pharmacological features. Some plants studied showed efficacy against gastric ulcers

such as: *Anacardium humile* have shown to possess antiulcerogenic activity (Luiz-Ferreira et al., 2010), as well as *Vernonia polyanthes* (Barbastefano et al., 2007), among others.

Protium heptaphyllum (Aubl.) Marchand, Burseraceae, is a medicinal plant largely found in the North and Northeast of Brazil, it is popularly known as "breu branco", and popularly used for inflammations, pain, ulcers and wounds. The triterpenes isolated from several species of medicinal plants are, in general, responsible, at least in part, for their biological activities (Aragão et al., 2006), including anti-inflammatory, anti-ulcer, anti-hyperlipidemic, anti-tumor, and hepatoprotective (Oliveira et al., 2005). Essential oils are complex mixtures comprising many single compounds, chemically derived from terpenes and their oxygenated compounds, these constituents contribute to the antifungal, antiviral, antioxidant effects (Laciar et al., 2009; Aidi et al., 2010). The present study aims to characterize the anti-ulcerogenic activity of the essential oil of *Protium heptaphyllum* March (BB) in three distinct induced gastric ulcer models: ethanol, Non Steroidal Anti Inflammatory Drugs and acetic acid as well as the mechanisms of action.

Materials and Methods

Animal

Male Wistar rats Unib: WH, (n=7, 180-250 g), were obtained from the Central Animal House of the State University of Campinas (CEMIB/Unicamp). Animals were fed a certified Nuvilab® (Nuvital) diet, with free access to tap water, and were housed on a 12 h light/dark cycle at 60±1% humidity and a temperature of 21±2 °C. The experimental protocols were all approved by the Institutional Committee for Ethics in Animal Experimentation of the Institute of Biology in the State University of Campinas (n° 852-1, CEEA/IB/Unicamp).

Essential oil

The BB was purchased from Laszlo aromaterapia Ltda. and was obtained from leaves and stem from this specimen by steam distillation. Parts of *Protium heptaphyllum* (Aubl.) Marchand, Burseraceae, were collected in the river delta Jequiriçá in the city of Valença, Bahia state (BA), Brazil by Antonio Calmon. An authenticated "voucher" specimen was identified by Jorge Yoshio Tamashiro of Unicamp and deposited under the number 151890 at UEC herbarium (Campinas, SP-Brazil).

Identification of essential oil constituents

The BB samples were analyzed in a gas chromatographer coupled to an electronic (70 eV) mass spectrometer (GC-MS, Shimadzu, GC-2010) equipped with a capillary column of fused silica (DB-5; 5.30 m x 0.32 mm x 0.25 µm), helium as the carrier gas (1.52 mL/min, White Martins, 99.9%), injector at 250 °C, detector at 250 °C and split injection mode. Mass spectrum acquisition was performed at a mass range from 40 to 600 *m/z*. The essential oil (10 µL) was diluted in chloroform to produce 1 mL of chromatographic grade solvent, 1 µL of which was injected as sample at the split ratio of 1:30. The column temperature was heated to 60 °C and programmed at 5 °C/min to 220 °C. The identification of substances was performed by the comparison of its mass spectra with the GC-MS system database (NIST 62 lib.), the literature and with the Kovats retention indices.

Drugs

The following drugs were used: lansoprazole (Medley, Campinas-SP, Brazil), Tween 80® and acetic acid (Sinth, SP, Brasil), absolute ethanol (©Merck KGaA, Darmstadt, Germany), cimetidine, carbenoxolone, indomethacin, were from Sigma Chemical Co. (St. Louis, USA). All reagents were of high grade of purity. The BB was dissolved in a 12% Tween 80® solution (w/v). The substances were prepared just before use.

Ethanol-induced gastric lesions

Ethanol-induced ulcers were evaluated in rats according to Morimoto (Morimoto et al., 1991). Rats fasted for 24 h were treated with BB (12.5, 25, 50 and 100 mg/kg), lansoprazole (30 mg/kg) or vehicle 12% Tween 80® (10 mL/kg). One hour after treatment all the animals received, orally, 1 mL/kg of absolute ethanol. Animals were killed by cervical dislocation one hour after ethanol administration, their stomachs removed, opened along the great curvature and fixed between two glass plates. The inner surface of the stomach was examined with a dissecting microscope (Nikon SMZ 800) and photographed with a Nikon Coolpix 4500 camera for later computer analysis. The total ulcerated area in the stomach corpus was measured with Bioview 4 (AvSoft, Brazil) an image analysis software (Khan, 2004).

Non steroidal anti-inflammatory drugs (NSAID) - induced gastric lesions

In this model, the gastric ulcer was induced using indomethacin (100 mg/kg), administered to rats after 36 h fast. BB (100 mg/kg), cimetidine (100 mg/kg) or vehicle 12% Tween 80 (10 mL/kg) was administered

orally, 30 min before the induction of gastric ulcer. The animals were killed by cervical dislocation 5 h after ulcer induction (Puscas et al., 1997). Their stomachs were removed and analyzed as previously described.

Chronic ulcer and toxicity evaluation

The experiments were carried out according to the method described by Takagi (Takagi et al., 1969) with some modifications in Okabe (Okabe & Amagase, 2005). Rats fasted for 24 h, under anaesthesia with ketamine and xylazine, a laparotomy was done in all animals through a midline epigastric incision. After exposing the stomach, one small tube was placed on the stomach, in the serosa region with an acetic acid solution 100% for 1 min. The abdomen was then closed and all the animals were fed normally. Two days after surgery, the treatment started: oral administration of BB (100 mg/kg), carbenoxolon (100 mg/kg) and vehicle Tween 80 12% (10 mL/kg), once a day for fourteen consecutive days. On the day after the last drug administration, the rats were killed by cervical dislocation, the stomachs removed and analyzed as described above. The toxicological parameters were set according to the method of Souza-Brito 1994 (Souza-Brito, 1994). The toxicity in the animals submitted to BB (100 mg/kg) treatment was evaluated for a period of fourteen days. The body weight progression, hair and mucosal alteration were observed daily. The following organs were weighed to detect any effect of the essential oil on their individual weights: heart, lungs, liver and kidneys.

Gastric secretion in pylorus ligature induced lesions

The method described by Shay (Shay et al., 1945), was used with some modifications. Rats were fasted for 36 h. Immediately after pylorus ligature, BB (100 mg/kg), cimetidine (100 mg/kg) and vehicle Tween 80 12% (10 mL/kg) were administered orally and intraduodenally. Rats were killed 4 h later, their abdomens were opened and their stomachs removed. The amount of the gastric juice (mL) and its pH were determined using a pH-meter (PA 200, Marconi S.A., Piracicaba, Brazil).

Western blot assay

Frozen glandular stomachs samples were homogenized in 1mL of ice cold phosphate buffer (PB 0.1M, pH 7.4 and protease inhibitor 1%). Homogenates were centrifuged (12000 g 15 min, 4 °C) and the supernatants were collected and stored at -80 °C. Protein concentration of the homogenate was determined following Bradford's colorimetric method (Bradford, 1976). Then, samples were treated with Laemmili buffer (PB 0.5 M, pH 6.8; glycerol, sodium dodecyl sulphate (SDS) 10%, bromophenol 0.1%, β-mercaptoethanol) in a 1:1 proportion. Equal amounts of protein from samples (100 µg) were separated on 10% acrylamide gel by sodium dodecyl sulphate polyacrylamide gel electrophoresis. In the next step, proteins were electrophoretically transferred onto a nitrocellulose membrane and incubated with specific primary antibodies: EGF (Santa Cruz Biotechnology, Inc, USA) and COX-2 (Cayman Chemical, USA) at dilution of 1:500. Each membrane was washed three times for 10 min and incubated with anti-goat immunoglobulin G antibody (Zymed Laboratories, USA) for EGF and with anti-rabbit (Zymed Laboratories, USA) for COX-2, both at dilution of 1:5000. To prove equal loading, the blots were analyzed for β-actin expression using an anti-β-actin antibody (Sigma-Aldrich, MO, USA). Immunodetection was performed using enhanced chemiluminiscence light-detecting kit (SuperSignal® West Femto Chemiluminescent Substrate, Pierce, IL, USA). Densiometric data were performed following normalization to the control (housekeeping gene) by AVSoft program.

Antioxidant activity

All tissues used in these antioxidant experiments were obtained from models of gastric ulcer induced by ethanol (Morimoto et al., 1991).

Glutathione peroxidase (GPx)

The activity of GPx in the gastric mucosa was evaluated spectrophotometrically at 365 nm. The absorbances were read at each minute between 1 and 10 min. This assay is based on the oxidation of the reduced glutathione by glutathione peroxidase coupled to the oxidation of NADPH by glutathione reductase. The tissue homogenate was diluted in phosphate buffer (PB) (1:10). 100 µL of this solution was mixed with 50µL H_2O_2 (0.25 mM), 20 µL of reduced glutathione (10 mM), 20 µL NADPH (4 mM), 10 µL (1 U) of glutathione reductase. The new solution was diluted again in PBS, pH 7.8 (Yoshikawa et al., 1993).

Glutahione redutase (GR)

GR activity was determined spectrophotometrically by measuring the rate of NADPH oxidation at 340 nm (Carlberg & Mannervick, 1985). Results are expressed as the amount of enzyme that catalyses the oxidation of 1 µmol of NADPH per minute per milligram of tissue (µmol/min^{-1}/mg $tissue^{-1}$).

Superoxide dismutase (SOD)

The SOD activity was assessed by the inhibition of the reduction of nitro blue tetrazolium (NBT) by the generated superoxide radical through the hypoxanthine/xanthine oxidase system (XO) at 37 °C. The enzymatic reaction was composed of: PB 0.1 M; pH 7.4; 0.07 U of XO mL; 100 µM hypoxanthine; 600 µM NBT and 1 mg/mL of protein from the sample (Winterbourn et al., 1995).

Mieloperoxidase (MPO)

The MPO activity in the gastric mucosa was determined according to the method of Winterbourn (Winterbourn et al., 1995). After the experiment of inducing ulcer with absolute ethanol, a portion of the glandular stomach of the animals was shaved and suspended in 1 mL of sodium phosphate buffer 0.05 M pH 6.8. The tissue was homogenized and centrifuged at 4 °C, 12000 x *g*, for 15 min and the supernatant was analyzed. The absorbances were read at 460 nm at each minute between 1 and 10 min. The results were expressed in U/g of protein.

Sulfhydryl group (GSH)

The samples were centrifuged (12000 x *g*, 4 °C for 15 min.) and the supernatant diluted (1:10) in sodium phosphate buffer (0. 1 M, pH 7.4). Then, a reading was made based on the absorbance of 100 µL of sample plus 100 µL of Tris solution (1.0 mM) and EDTA (0.02 mM) at 412 nm (A1). After the reading, 20 µL of (5.5 ditiobis 2-nitrobenzoic) acid (DTNB 0.01 mM) was added, dissolved in methanol and has been re-read (A2) at 412 nm after 15 min. of reaction. The concentration of sulfhydryl group (thiol) is given by (A1-A2) x 1.57 (Faure & Lafond, 1995).

Determination of lipid peroxidation or malondialdehyde (MDA) formation

The concentrations of gastric mucosal lipid peroxidation were determined by estimating malondialdehyde using the thiobarbituric acid test (Ohkawa et al., 1979). The stomachs of the rats were promptly excised and rinsed with cold saline. To minimize the possibility of hemoglobin's interference with free radicals, any blood adhering to the mucosa was carefully removed. The gastric mucosa was scraped, weighed, and homogenized in 10 mL of 100 g/L KCl. The homogenate (0.5 mL) was added to a solution containing 0.2 mL of 80 g/L sodium lauryl sulfate, 1.5 mL of 200 g/L acetic acid, 1.5 mL of 8 g/L 2-thiobarbiturate, and 0.3 mL of distilled water. This mixture was heated at 98 °C for 1 h. and after cooled, 5 mL of *n*-butanol:pyridine (15:l) was added. The mixture was vortexed for 1 min. and centrifuged for 30 min. at 4000 x *g*. The supernatant absorbance was measured at 532 nm. The standard curve was obtained by using 1,1,3,3-tetramethoxypropane. The recovery was over 90%. The results were expressed as nanomoles of MDA per gram of wet tissue (nmol/g tissue).

Statistical analysis

Results were expressed as the mean±SEM. Statistical significance was determined by one-way analysis of variance (ANOVA) followed by Dunnett′s or Tukey's post-hoc test, with the minimum level of significance set at $p<0.05$.

Results and Discussion

Although there are many products in the market for the treatment of gastric ulcers, including antacids, proton pump inhibitors, anticholinergics and histamine H2-antagonists, most of these drugs produce several adverse reactions, such as gynecomastia, hematopoietic changes, acute interstitial nephritis, thrombocytopenia anaphylaxis reactions, nephrotoxicity and hepatotoxicity. In developing countries the peptic ulcer treatment is very expensive, thus, there is a growing need for less expensive antiulcer agents with minor side effects. In this context, medicinal plants are amongst the most attractive sources of new drugs, and have been shown to give promising results in the treatment of gastric ulcers. In Brazil, a large number of herbal extracts is used in folk medicine to treat various types of digestive disorders (Hiruma-Lima et al., 2006). We studied the gastroprotective effect of the BB and its possible mechanisms of action.

In the present investigation, the GC-MS analysis revealed that the components of the BB are α-pinene 40%, followed by *p*-mentha-1.4(8)-diene 12% and α-phellandrene 10% and thirteen components in lower concentrations (Table 1), thus revealing that this oil is composed mainly by monoterpenes. Monoterpenic essential oils are considered natural antioxidants due to the presence of α-pinene (Singh et al., 2010; Aidi et al., 2010).

Oral administration of absolute ethanol is noxious to the stomach since it affects the gastric mucosa topically by disrupting its barrier and provoking pronounced microvascular changes within a few minutes after its application (Moleiro et al., 2009). Even oral administration of absolute ethanol to rats produced linear hemorrhagic lesions, extensive submucosal edema, mucosal friability, inflammatory cells infiltration, and epithelial cell loss in the stomach, which are typical characteristics of alcohol injury

(Franke et al., 2005). The pathogenesis of ethanol-induced gastric mucosal damage occurs directly and indirectly through various mediators such lipoxygenase, cytokines, and oxygen-derived free radicals (Abdel-Salam et al., 2001). Oral administration of BB (12.5, 25, 50 and 100 mg/kg) inhibited ethanol-induced gastric lesions by 4, 45, 46 and 96% respectively. When compared to the control value, BB significantly inhibited ulcerative lesions at the dose of 100 mg/kg, therefore this dose was used in all other experiments (Table 2).

Table 1. Chemical composition (percentage) of essential oils extracted from *Protium heptaphyllum* using analysis by GC-MS.

Peak	RT	Compound	Composition%
1	4,283	α-thujene	1,03
2	4,435	α-pinene	40,32
3	4,734	camphene	1,32
4	5,311	sabinene	0,12
5	5,376	β-pinene	4,78
6	5,448	*p*-menth-2-ene	0,1
7	5,765	β-myrcene	0,11
8	5,919	carane	2,36
9	6,062	α-phellandrene	10,27
10	6,208	3-carene	5,78
11	6,377	2-carene	2,31
12	6,582	*p*-cimene	9,64
13	6,696	*m*-mentha-1,8-diene	8,88
14	7,518	δ-terpinene	0,66
15	8,322	*p*-mentha-1,4(8)-diene	12,14
16	9,841	camphor	0,18

RT- Retention time

Table 2. Effect of BB on gastric lesions induced by ethanol and indomethacin in rats. Results were expressed as mean±SEM. Data were analyzed by ANOVA followed by Tukey's test and compared to vehicle. *p<0.01 **p<0.001.

Treatments (*p.o.*)	Dose (mg/kg)	Damage Area (mm^2)	Gastroprotection %
Ethanol-induced gastric lesions			
Vehicle	10	104.3±19.3	-
Lansoprazole	30	40.5±5.9*	38.8
BB	12.5	99.9±6.9	4.2
	25	56.7±11.4	54.3
	50	56.4±20.0	54.0
	100	4.1±1.0**	96.0
Indomethacin-induced gastric lesions			
Vehicle (mL/kg)	10	29.9±13.5	-
Cimetidine	100	0.9±0.4**	96.9
BB	100	0.9±0.1**	96.9

It is well known that indomethacin is used not only as an anti-inflammatory drug but also to induce an experimental ulcer model in rats (Polat et al., 2010), due to the fact that this compound inhibits the synthesis of cytoprotective prostaglandins, synthesized by COX-1 and COX-2 in the stomach tissue. Furthermore, it's also been shown that reactive oxigen species (ROS) play an important role in the pathogenesis of mucosal damages caused by indomethacin (Odabasoglu et al., 2006). Oral treatment with BB (100 mg/kg) reduced the indomethacin- induced gastric lesion by 95% (Table 2).

Ulcer healing, a genetically programmed repair process, includes inflammation, cell proliferation, reepithelialization, formation of granulation tissue, angiogenesis, interactions between various cells and the matrix and tissue remodeling, all resulting in scar formation, the capacity to accelerate the ulcer healing process depends on many factors, like the epidermal growth factor (EGF), fibroblast growth factor (bFGF), vascular endothelial growth factor (vEGF), trefoil peptides and COX-2 in a well synchronized spatial and temporal manner (Tarnawski, 2005). Regarding the COX-2 (Figure 1A) and EGF (Figure 1B) expression, Western Blotting analysis in the present study shows a great quantity of both in the gastric mucosa of animals treated with BB.

It has been shown that COX-2 induced in ulcerated gastric mucosa is involved in the defense and repairing mechanisms of the mucosa and that its inhibition by a selective COX-2 inhibitor delays ulcer healing, we have also shown, in human stomach, that COX-2 is exclusively expressed in gastric mesenchymal cells such as fibroblasts and in inflammatory cells of the ulcer bed and margins, suggesting that COX-2 expressed in mesenchymal cells at the ulcer margin plays a key role in the ulcer repair process (Miura et al., 2004).

Several authors associate the antiulcerogenic process with healing of chronic ulcers and participation of EGF. Growth factors and their receptors play important roles in cell proliferation and migration, repair of the tissue injury and ulcer healing, considering that EGF is effective at protecting the gastric mucosa from acetic acid-induced gastric lesions, it is reasonable to assume that this factor limits mucosal damage caused by ulcerogenic agents, probably aiding early mucosal restoration (Konturek et al., 1992). The increase in COX-2 (Figure 1A) and EGF (Figure 1B) expression in rats treated with BB may indicate a participation of this protein in restoring the gastric mucosa.

Acetic acid induces gastric ulcers by a perforative nature, spread over a large area that does not heal with time, the application of glacial acetic acid to the gastric serous membrane caused ulcer with

wall-encircled deep craters (Hiruma-Lima et al., 2006). Postoperative treatment with BB (100 mg/kg) for fourteen consecutive days demonstrated, for the first time, that BB accelerated ulcer healing. On the day 14 after surgery, the percentage of rats with cicatrized ulcers in both experimental groups was significantly higher than that of the control group. In addition, BB (100 mg/kg) significantly decreased the mean area of chronic ulcer (Figure 2).

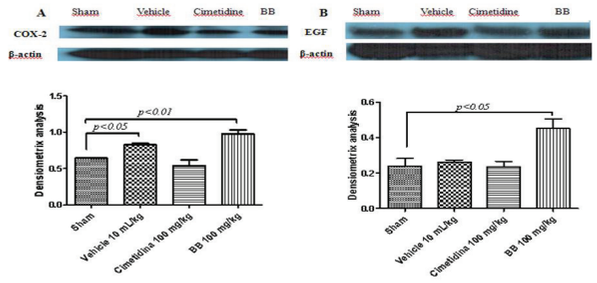

Figure 1. Representative Western blot analysis COX-2 (A) and EGF (B) proteins. Densitometric data were studied following normalization to the control (β-actin house-keeping gene). The results are representative of three experiments performed on different samples and data are expressed as mean±SEM.

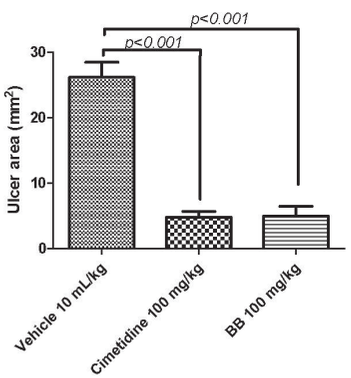

Figure 2. Effects of chronic administration of BB 100 mg/kg on ulcer healing in rats with chronic ulcer induced by 0.05 mL (v/v) of a 30% acetic acid solution. This dose significantly decreased the severity and extent of area damaged by acetic acid at fourteen days. Results were expressed as mean ±SEM. Data were analyzed by ANOVA followed by Tukey's test and compared to vehicle.

On toxicological parameters, there were no significant differences in body weight development (data not shown) or organ weights (Figure 3) for all groups. No macroscopic abnormalities were detected in the examined organs. No mortality observed in any treatment group during the fourteen days of study.

Ligation of the pylorus model produces accumulation of gastric juice with gastric acid hypersecretion which generates the wound in the mucosa. The hypersecretion of gastric acid is one of the major pathogenic factors of gastric ulcer (Hiruma-Lima et al., 2009; Tuorkey & Karolin, 2009). The gastric juice obtained from pylorus-ligated rats was used to analyze the gastric biochemical parameters by oral or intraduodenal BB administration. Administration of BB (100 mg/kg) by different routes (oral and intraduodenal) showed no significant difference in the assessed parameters of the gastric juice (data not shown). Thus, an antisecretory activity may not be involved in this protection.

Intracellular antioxidant, such as GR is critical for cellular protection in gastric tissues, GSH and GR have a prominent role in tissue repairing when reactive oxygen species (ROS) are involved, it was reported that, in humans, a reduction in gastric GR can occur following ethanol consumption and GR pretreatment could subside the gastric damage (Morais et al., 2010). It was also reported that an increase in GSH levels show a parallel to the adaptation phenomenon, the higher the GSH level, the less the damage occurred, GSH and other antioxidants prevented tissue damage by keeping ROS at physiological levels (Polat et al., 2010). The results indicated that GSH (Figure 4) and GR (Table 3) levels increased if compared to the control group, demonstrating a possible antioxidant capacity of BB.

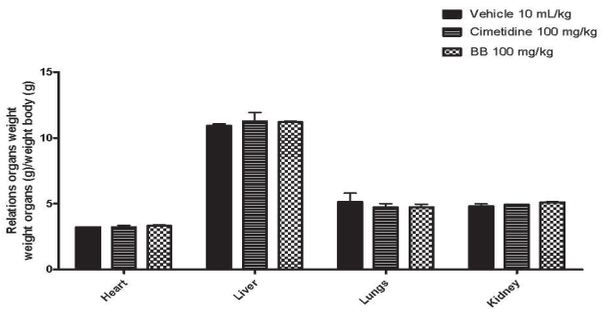

Figure 3. Organs weight in rats treated orally with vehicle, lansoprazole (30 mg/kg) or BB (100 mg/kg) for fourtenn days after ulcer formation by acetic acid solution injected into the stomach.

MPO exists in polymorphnuclear leukocyte cells (PNL) and catalyses the formation of toxic hypochlorous acid (HOCl) from hydrogen peroxide, in addition, PNL excessively produce superoxide anion (O_2^-) and hydroxyl radical (OH^-), which are free oxygen radicals, excessive production of MPO and other reactive radicals cause oxidative damage; which is represented by measuring lipid peroxidation levels, an important reason for cell membrane damage; MDA is the final product of lipid peroxidation and is used to determine lipid peroxidation levels (Dursun et al., 2009). Gastric MPO (Figure 5) and MDA (Figure 6) were increased by indomethacin application and decreased by BB (100 mg/kg) administration, another indicator of a possible antioxidant activity of the oil, which still needs deeper studies for a complete understanding.

Ethanol may enhance the damage associated with the increased steady-state levels of ROS and could act by increasing the activity of SOD. Previous studies have shown SOD activity increased values in the ethanol treated group compared to control rats, suggesting that oxidative stress condition can increase the levels of O_2^- or induce the activity of the enzyme (Repetto et al., 2003). Some researchers reported that ethanol increases GPx activity (Suleyman et al., 2010). The BB maintained the same levels of SOD (Table 3) and GPx (Table 3) of the sham group, this effect suggests that BB could decrease the oxidative stress condition generated by ethanol.

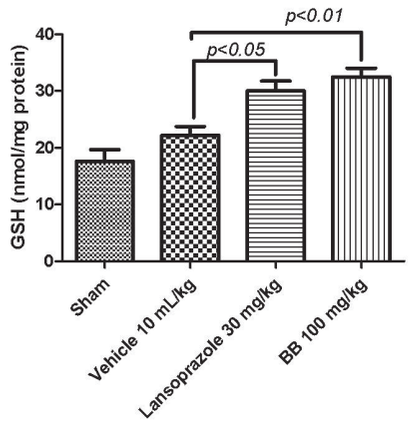

Figure 4. Sulfhydryl contents in the gastric mucosa of rats pretreated with BB (100 mg/kg) and submitted to absolute ethanol-induced ulcerative lesions. Results were expressed as mean±SEM. Data were analyzed by ANOVA followed by Tukey's test and compared to vehicle.

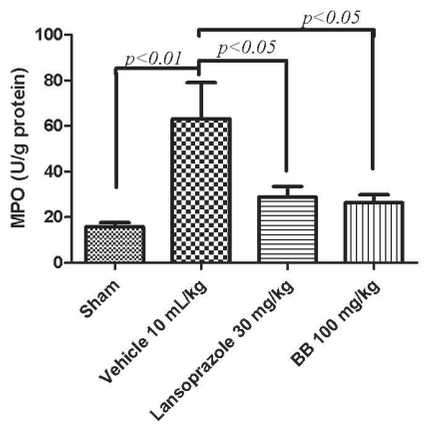

Figure 5. Effect of BB (100 mg/kg) on MPO activity in the gastric mucosa of rats submitted to ethanol. Results were expressed as mean±SEM. Data were analyzed by ANOVA followed by Tukey's test and compared to vehicle.

Conclusion

In conclusion, all these results taken, together, show, for the first time, that BB exerts gastroprotective activity, as evidenced by the significant inhibition of the formation of ulcers induced by different models, these protection could be attributed to an increased COX-2 and EGF expression and to the antioxidant properties. This antioxidant activity could be influenced by the presence of monoterpenes in the chemical structure of BB. However, further studies are required to investigate the active compounds and elucidate the mechanisms involved in the effects.

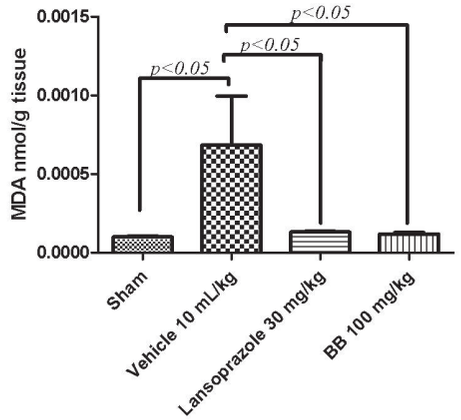

Figure 6. Effect of BB (100 mg/kg) on levels of MDA in ethanol-treated rats. Results were expressed as mean±SEM Data were analyzed by ANOVA followed by Tukey's test and compared to vehicle.

Table 3. Effect of BB (100 mg/kg) on stomach antioxidants enzymes and the levels of MDA in ethanol-treated rats. Results were expressed as mean±SEM. Data were analyzed by ANOVA followed by Tukey's test and compared to vehicle. *p<0.05.

Treatments	SOD (U/mg protein)	GPx (nmol/min/mg of protein)	GR (mmol/min/mg of protein)
Sham	1.9±0.2	1.8±0.3	0.9±0.1
Vehicle	3.4±0.5	3.1±0.3	0.9±0.1
Lansoprazole	2.2±0.1*	1.9±0.2*	1.5±0.1*
BB (100 mg/kg)	2.0±0.1*	1.8±0.2*	1.6±0.1*

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