academicJournals

Vol. 11(39), pp. 603-612, 17 October, 2017
DOI: 10.5897/JMPR2017.6436
Article Number: 1349A8666324
ISSN 1996-0875

http://www.academicjournals.org/JMPR

Journal of Medicinal Plants Research

Full Length Research Paper

Gastroprotective effect of the aqueous fraction of hydroacetonic leaf extract of *Eugenia uniflora* L. (Myrtaceae) (pitanga) against several gastric ulcer models in mice

José Luís Rodrigues M.[1*], Dayane Moreira da S.[2], James Oluwagbamigbe F.[2], Emerith Mayra Hungria P.[1], Eric de Souza G.[3], Anderson Luiz F.[2], Suzana da Costa S.[4] and Elson Alves C.[2]

[1]Faculty of Pharmacy, University Center of Anápolis - Unievangélica, University Avenue, km 3,5, University City, s/n, 75083-515 Anápolis-GO, Brazil.
[2]Department of Pharmacology, Institute of Biological Sciences, Federal University of Goiás, Campus Samambaia, Campus Street, s/n, 74001-97, Goiânia-GO, Brazil.
[3]Institute of Chemistry, Federal University of Goiás, Campus Samambaia, Goiânia-GO, Brazil.
[4]Institute of Chemistry, Federal University of Goiás, Brazil.

Received 16 June, 2017; Accepted 11 September, 2017

***Eugenia uniflora* L. (Myrtaceae) is popularly known in Brazil as pitanga or ibitanga. The infusion of *E. uniflora* leaves is being used in folk medicine as anti-diarrheal. The present study sought to evaluate the gastroprotective potential of the aqueous fraction of hydroacetonic leaf extract of pitanga (AFHP). The leaf powder of pitanga was extracted with 50% acetone using an overhead stirrer apparatus at room temperature in which the acetone was removed under reduced pressure and the suspended aqueous. The aqueous layer was freeze-dried to yield a 122 g aqueous fraction, which was stored at -20°C. Preliminary investigation showed that AFHP (100, 300 and 1000 mg/kg, p.o.) is devoid of any behavioral neurotoxic signs. The anti-ulcer activity of AFHP was evaluated in the gastric ulcer models induced by indomethacin, stress and HCl/EtOH in mice. In order to identify possible mechanisms of gastroprotective activity of AFHP, antisecretory activity of this fraction was conducted. The quantification of adhered gastric mucus reduced glutathione (GSH) and the role of nitric oxide (NO) were also investigated. The AFHP showed antiulcer activity in various models of acutely induced ulcers. The intra-duodenal administration of this fraction reduced total acidity and increased pH of the gastric secretion. Oral administration prevented a decrease in the amount of adhered mucus and increased GSH levels. Pretreatment with L-NAME did not affect the gastroprotective effect of AFHP. Our results suggest that AFHP exhibits antiulcer activity that involved an increased in gastric mucus and in the levels of GSH.**

Key words: *Eugenia uniflora*, pitanga, myrtaceae, gastric ulcer, gastroprotection, mucus.

INTRODUCTION

The peptic ulcer disease (PUD) is one of the most common disorders of gastrointestinal tract (TGI) with a prevalence of 4 to 5% in human society (El-Maraghy et al., 2015). The PUD is a gastrointestinal disorder that

occurs in the stomach and duodenum and generally is caused by an imbalance between aggressive and protective factors (Santin et al., 2010). It is well known that the major etiological factors involved in the onset of peptic ulcers are *Helicobacter pylori* infection, prolonged use of non-steroidal anti-inflammatory drugs (NSAIDs), ischemia of the gastric mucosa, age, genetic factors, stress, alcohol, smoking and dietary habits (Caldas et al., 2011).

Medicinal plants are known as an important source of compounds for the treatment of gastric ulcers and new drugs discovery (Borrelli and Izzo, 2000; Zanatta et al., 2009). Several authors have shown that different species from Myrtaceae family such as *Campomanesia xanthocarpa* O. Berg (Markman et al., 2004), *Eugenia jambolana* (Chaturvedi et al., 2007; El-Shenawy, 2009), *Myrtus communis* L. (Sumbul et al., 2010), *Plinia edulis* (Vell.) Sobral (Ishikawa et al., 2008), *Eugenia dysenterica* DC. (Prado et al., 2014) and *Eugenia punicifolia (*Kunth) DC. (Basting et al., 2014) among others, have gastroprotective activity.

Eugenia uniflora L. (Myrtaceae) is popularly known as cherry, ibipitanga, pitanga or naganpiri (Consolini and Sarubbio, 2002; Rattmann et al., 2012; Weyerstahl et al., 1988). This species of bushy plant with edible fruit is native to Brazil and widely distributed in South America countries (Lorenzi and Souza, 1999). The fruits are rich in calcium, anthocyanins, flavonoids, carotenoids and vitamin C that conferred high antioxidant property of this species. The pleasant flavor and odor make this specie a desirable content of ice cream, juices, jams, wines and cosmetics (Lima et al., 2002; Lopes, 2008). The *E. uniflora* leaves are rich in tannins and flavonoids (Auricchio and Bacchi, 2003) and several studies have shown that tannin rich species have been traditionally used for their gastroprotective effects (de Jesus et al., 2012).

The folk medicine reports the use of hydro-alcoholic extract of *E. uniflora* leaves to control the levels of triglycerides, very low-density lipoproteins (VLDL) cholesterol and uric acid (Ferro et al., 1988). Furthermore, the use of *E. uniflora* leaves as anti-inflammatory, diuretic (Schapoval et al., 1994), antispasmodic (Amorim et al., 2009), antihypertensive (Consolini and Sarubbio, 2002), bactericidal, cytotoxic (Bouzada et al., 2009), anti-candida activity (Santos et al., 2013), anti-*Trypanosoma cruzi* activities (Santos et al., 2012) antidepressant-like effect (Victoria et al., 2013) and antidiarrheal (Almeida et al., 1995; Victoria et al., 2012) have also been reported. The objective of the present study was to evaluate the potential gastroprotective activity of the aqueous fraction of hydroacetonic leaf extract of the pitanga (AFHP).

MATERIALS AND METHODS

Botanical material

The *E. uniflora* leaves were collected at Anápolis, Goias, Brazil (16°20' 12.8 "S, 48°56' 19.3" W). The botanical material was authenticated by Prof. Dr. Heleno Ferreira Diaz of the Botany Department, Federal University of Goiás. A specimen voucher was deposited at the herbarium of the Federal University of Goiás (n°. 25477). The concentrations of AFHP and drugs were adjusted to ensure that all treatments respect the volume of 10 mL/kg in purified water for oral treatment or in saline for i.p administrations.

Preparation of aqueous fraction of hydroacetonic leaf extract

Dried and ground leaves of *E. uniflora* (1.0 kg) were exhaustively extracted with 50% acetone, using an overhead stirrer apparatus at room temperature. The acetone was removed under reduced pressure and the suspended aqueous extract was filtered to eliminate fats and chlorophylls. Following, a liquid-liquid extraction with ethyl acetate (10 × 150 mL) was carried out. The combined organic phase was evaporated to yield an ethyl acetate fraction (15 g). The aqueous layer was freeze-dried to yield a 122 g aqueous fraction, which was stored at -20°C.

Animals

Swiss albino male mice, weighing 25 to 35 g from the Central Animal Laboratory of the Federal University of Goiás were used. The animals were housed at 22±1°C on a 12 h (h) light/dark cycle with free access to food and water. All experiments were approved by the Ethics Committee on Animal Use (Protocol number 038/14).

Drugs and chemicals

The drugs and chemicals used included: ethanol (EtOH) (Quimex, São Paulo, SP, Brazil), carbenoxolone, L-Name (NG-nitro-L-arginine), indomethacin and Alcian blue (Sigma Chemical Company, St. Louis, MO, EUA); ranitidine (Teuto, Anápolis, GO, Brazil), sacarose (Lafan, Varzea Paulista, SP, Brazil), magnesium chloride (Quimibras, Rio de Janeiro, RJ, Brazil), sodium acetate (Vetec, Duque de Caxias, RJ, Brazil), sodium hydroxide (Lafan, Varzea Paulista, SP, Brazil), Ethylenediaminetetraacetic acid (EDTA), 5,5-ditiobis-2-nitrobenzoic acid (DTNB), trichloroacetic acid (TCA), and reduced glutathione (GSH).

General pharmacological activity test

The groups of animals (n = 3) were treated orally or intraperitoneally with increasing doses of AFHP (100, 300 or 1000 mg/kg), while the control group received vehicle (distilled water 10 mL/kg, p.o.) or saline (10 mL/kg, i.p.). After the treatments, the animals were observed for 3 min under free ambulation on the flat surface after 5, 10, 20, 30 and 60 min; 4, 8, 24 and 48 h; 4 and 7 days of treatment. The observed behavioral changes that differentiate treated animals of the control group were reported in standard pharmacological screening form (supplementary Table 1) adapted from Irwin proposal (Irwin, 1968).

*Corresponding author. E-mail: jlfarmacia@hotmail.com.

Indomethacin-induced ulcer

After 16 h of fasting, animals (n = 8/group) were orally treated with vehicle (10 mL/kg), AFHP (100, 300 and 1000 mg/kg) or ranitidine (50 mg/kg). After 60 min of treatment, all animals received indomethacin (30 mg/kg, s.c.) and after 3 h from the administration of the ulcerogenic agent, all treatments were repeated. Animals were euthanized 6 h after the administration of indomethacin to removed the stomachs for the evaluation of lesion index (LI) (Djahanguiri, 1969). The LI and the percentage of gastric ulcer inhibition were calculated according to Rios et al. (2010) (Table 1).

Hypothermic restraint stress ulcer

After 16 h of fasting, the animals (n = 8/group) received vehicle (10 mL/kg, p.o), AFHP (300 mg/kg, p.o) or ranitidine (50 mg/kg, p.o). One hour after treatment, gastric ulceration was induced by immobilizing the animals in a closed cylindrical cage maintained at 4°C. After 2 h, the mice were euthanized to remove the stomach for LI assessment (Senay and Levine, 1967).

Ethanol/HCl-induced ulcer

After 16 h of fasting, the animals (n = 8/group) received vehicle, AFHP (300 mg/kg) or carbenoxolone 200 mg/kg by gavage. One hour after treatment, all the animals received 0.3 M HCl/ethanol 60% solution (10 mL/kg, p.o.) orally to induce acute gastric lesions (Caldas et al., 2011).

The animals were euthanized 1 h after induction of gastric lesions, while the stomachs were removed and opened along the greater curvature. The stomachs were photographed and the area of lesions (%) was measured by AUTOCAD software.

Parameters involved in gastric acid secretion

The pylorus ligature was performed by adaptation of method described by Shay et al. (1945). After 16 h of fasting, animals (n=8/group) were anesthetized and pylorus ligature was carried out. Mice received vehicle, AFHP (300 mg/kg) or ranitidine (50 mg/kg) intraduodenally (i.d.). Four hours later the animals were sacrificed, the stomachs were removed and the gastric luminal contents were centrifuged for 30 min at 2000 *g.* The supernatant was used to measure the gastric juice volume (mL), total acidity and pH.

Quantification of gastric wall adhered mucus

The modified method of Corne et al. (1974) was used to quantify gastric mucus. After 16 h of fasting, the animals (n=8) received vehicle, AFHP (300 mg/kg) or carbenoxolone (200 mg/kg) by gavage. After 60 min, all groups were orally treated with 60% ethanol solution (10 mL/kg, p.o.). The contents of the stomach was weighed and transferred to a test tube containing 7 mL of 0.1% Alcian blue (0.16 M sucrose in 0.05 M sodium acetate, pH 5.8). After two consecutive rinses with 5 mL of sucrose (0.25 M), 5 mL of $MgCl_2$ (0.5 M) was added in each test tube for the extraction of mucus content with the dye. The glandular segment remained in this solution for 2 h with intermittent agitation. After which 4 mL of the resultant blue solution was agitated vigorously with 4 mL of ethyl ether until the formation of an emulsion and was centrifuged for 10 min at 3600 *g*. The absorbance of the supernatant was measured at 598 nm using a spectrophotometer. The concentration of Alcian blue was calculated through a calibration curve and the results were expressed in μg of Alcian blue/g of glandular tissue.

Quantification of reduced glutathione (GSH)

After ethanol induced gastric ulcer, the other half segment of the glandular stomach area was weighed and transferred to a tube where the homogenate was done with ice-cold 0.02 M ethylenediaminetetraacetic acid 10% (EDTA). According to the method proposed by Sedlak and Lindsay (1968), 400 µL aliquots of homogenate were mixed with 320 µL of distilled water and 80 µL of 50% trichloroacetic acid in Eppendorf tubes and centrifuged at 3000 g for 15 min. Subsequently, the supernatant (400 µL) was mixed with 800 µL Tris HCl (0.4 M, pH 8.9) and 5, 5'-dithiobis-2-nitrobenzoic acid (DTNB 0.01 M). The absorbance was read within 3 min at 412 nm. The concentration of GSH was calculated using a standard curve of reduced glutathione (GSH) expressed in μg /g of tissue.

HCl/EtOH-induced gastric mucosa ulcer in mice pretreated with L-NAME

This method was performed as described by Matsuda et al. (1999). After a 16 h of fasting, the animals were pretreated with 0.9% saline (10 mL/kg, i.p.) or L-NAME, an inhibitor of NO synthase (20 mg/kg, i.p.). Thirty minutes later, animals received an oral dose of vehicle or AFHP (300 mg/kg). After 60 min, all groups were orally treated with 0.45M HCl/60% ethanol solution (10 mL/kg) to induce gastric-ulcer. After 1h, animals were euthanized and the stomachs were removed, opened along the greater curvature and gastric damage was determined as described above.

Statistical analysis

Results were expressed on means ± standard error of mean (SEM) absolute or percentage values and were compared using one-way analysis of variance (ANOVA) followed by the Tukey post-hoc test (to analyze more than two groups) or student unpaired "t" test (to analyze two independent groups (Drummond and Tom, 2011a, b). Effects were considered significant at $p < 0.05$.

RESULTS

Effects of AFHP in the general pharmacological evaluation

AFHP at doses of 100, 300 and 1000 mg/kg administrated intraperitoneally (i.p.) caused a reduction of spontaneous movements and induced writhing which was observed between 5 min to 4 h. The dose of 100 mg/kg caused analgesia and alienation from 5 min until 4 h at a dose of 300 to 1000 mg/kg and in addition to the changes described above catatonia, ataxia, diarrhea were also observed. Death of animals was recorded at the highest dose (i.p) within 24 h. The animals treated with different doses by oral route showed no behavioral changes that differentiate animals treated with vehicle (supplementary Table 1).

Effect of AFHP in gastric ulcer induced by non-steroidal anti-inflammatory drug (NSAID)

The administration of indomethacin (NSAID) produced

Table 1. Score attribution scale for the degree of ulceration.

Index of lesion	Score
Discoloration of mucosa	1
Edema	1
Hemorrhages	1
Number of petechia	
Until 25%	2
More than 25%	3
Intensity of ulceration	
Ulcers or erosion up to 1 mm	*N* × 2
Ulcers or erosion larger than 1 mm	*N* × 3
Perforated ulcers	*N* × 4

N, Number of stomach lesions.

Table 2. Effects of AFHP or ranitidine on indomethacin induced ulcers in mice.

Group	Treatment (p.o.)	Dose (mg/kg)	LI	Reduction (%)
Control	Vehicle	-	8.7±0.7	-
Ranitidine	Ranitidine	50	5.1±0.3***	41.7
		100	5.9±0.5***	32.7
AFHP	AFHP	300	5.3 ±0.3***	38.7
		1000	5.0±0.5***	42.9

Results are expressed as mean ± SEM of the LI for eight mice. Statistical comparison was performed using ANOVA followed by the Tukey. ***p ≤ 0.001 compared with the control group.

extensive lesions in the gastric mucosa. Treatment of mice with AFHP (100, 300 or 1000 mg/kg) significantly reduced the LI (Table 2).

Effect of AFHP in gastric ulcer induced by hypothermic restraint stress

In the gastric ulcer induced by stress in the model of hypothermic restraint, treatment with AFHP or ranitidine significantly reduced the LI when compared with the control group (Table 3).

Effect of AFHP on gastric ulcer induced by HCl / ethanol in mice

Administration of HCl/ethanol yielded extensive lesions in the gastric mucosa of the stomach. These lesions were characterized by multiple red or dark brown spots of different sizes along the gastric mucosa (Figure 1). Treatment of mice with AFHP significantly reduced the ulcerated area by 58.9% when compared to the control group (Table 4).

Effect of AFHP on parameters of gastric acid secretion in mice

The treatment with AFHP (300 mg/kg) was unable to decrease the volume of gastric secretion. However, AFHP increased the pH and decreased the total acidity. The treatment with ranitidine (50 mg/kg) caused a decrease in the volume of gastric acid secretion, total acidity and increased pH (Table 5).

Effect of AFHP on gastric adhered mucus

The alcian blue binding capacity of gastric mucus in the control group with lesion (ethanol 60%, 10 mL/kg, p.o.) was significantly reduced compared with the control group without injury. However, the groups of animals with lesions that were pretreated with AFHP or carbenoxolone significantly increased the alcian blue binding capacity of gastric wall mucus (Table 6).

Effect of AFHP on the amount of GSH in the stomach tissue

The GSH content in the control group with lesion (EtOH

Table 3. Effects of AFHP or ranitidine on stress-induced gastric lesions in mice.

Group	Treatment (p.o.)	Dose (mg/kg)	Index of lesion	Reduction (%)
Control	Vehicle	-	9.1±1.0	-
Ranitidine	Ranitidine	50	5.1±0.3***	43.8
AFHP	AFHP	300	5.1±0.2***	43.8

Results are expressed as mean ± SEM of the LI for eight mice. Statistical comparison was performed using ANOVA followed by the Tukey. *** $p \leq 0.001$ compared with the control group.

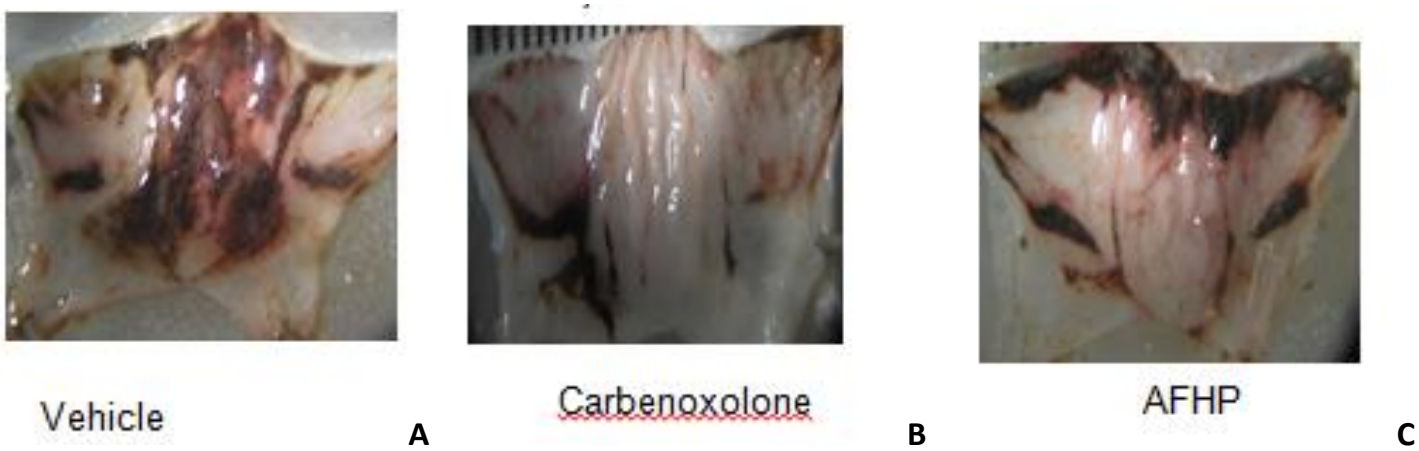

Figure 1. Gastroprotective effects of AFHP or carbenoxolone on the HCl/ethanol-induced gastric lesion in mice.

Table 4. Effects of AFHP or carbenoxolone on HCl/ethanol-induced gastric lesions in mice.

Group	Treatment (p.o.)	Dose (mg/kg)	Ulcerated area (%)	Reduction (%)
Control	Vehicle	-	19.00±3.86	-
Carbenoxolone	Carbenoxolone	200	2.05±0.49***	89.2
AFHP	AFHP	300	7.80 ±2.3**	58.9

Results are expressed as mean ± SEM of the ulcerated area (%) for eight mice. Statistical comparison was performed using ANOVA followed by the Tukey ** $p \leq 0.01$ *** $p \leq 0.001$ compared with the control group.

Table 5. Effects of AFHP or ranitidine extract, administered intraduodenally, on the biochemical parameters of gastric juice obtained from pylorus ligature in mice.

Group	Treatment (i.d.)	Dose (mg/kg	Volume (ml)	pH	Gastric acidity (mEq[H^+] /L/4h)
Control	Vehicle	-	2.37±0.05	3.25±0.2	4.34±0.5
Ranitidine	Ranitidine	50	2.12±0.04*	3.69±0.05 **	1.79±0.07***
AFHP	AFHP	300	2.20±0.07	4.20±0.08***	2.40±0.3**

Results are expressed as mean ± SEM for eight mice. Statistical comparison was performed using ANOVA followed by the Tukey. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$ compared with the control group.

60%, 10 mL/kg, p.o.) was significantly reduced compared with control group without injury. However, the groups of animals with lesions that were pretreated with AFHP or carbenoxolone significantly increased the GSH content in 48.24 or 11.84%, respectively, when compared with control group with lesion (Table 6).

Effect of AFHP on HCl/ethanol-induced gastric mucosal lesion with L-NAME-pretreated mice

Treatment with HCl/EtOH induced extensive lesions in the gastric mucosa of the stomach. However, treatment with AFHP significantly reduced the ulcerated area in

Table 6. Effect of oral treatment of AFHP or carbenoxolone on the gastric adhered mucus and GSH in the model of ethanol (60 %, 10 mL/kg, p.o.) in mice.

Group	Treatment (p.o.)	Dose (mg/kg)	Alcian blue (µg/g tissue)	GSH (µg/g tissue
Control without lesion	Vehicle + water	-	38.4±2.3	150.4±5.6
Control with lesion	Vehicle + EtOH	-	29.1±2.1*	128.0±3.0**
Carbenoxolone	Carbenoxolone + EtOH	200	41.0±1.4$^{\#\#}$	143.5±3.8$^{\#}$
AFHP	AFHP + EtOH	300	37.5±1.7$^{\#}$	190.2±11.8$^{\#\#}$

Results are expressed as mean ± SEM for eight mice. Statistical comparison was performed using ANOVA followed by the Tukey test. * $p \leq 0.05$, ** $p \leq 0.01$, control without lesion vs control with lesion; $^{\#}$ $p \leq 0.05$, $^{\#\#}$ $p \leq 0.01$, carbenoxolone or AFHP vs control with lesion.

Table 7. Role of nitric oxide (NO) in the gastroprotective effect of AFHP against HCl/EtOH-induced gastric injury in mice.

Pretreatment (i.p.)	Treatment (p.o.)	Ulcerated Area (%)
Saline 10 mL/kg	Vehicle 10 mL/kg	64.8±3.3
Saline 10 mL/kg	AFHP 300 mg/kg	23.2±1.9***
L-Name 20 mg/kg	Vehicle 10 mL/kg	68.1±5.0
L-Name 20 mg/kg	AFHP 300 mg/kg	16.5±3.5***

Results are expressed as mean ± SEM for eight mice. Statistical comparison was performed using ANOVA followed by the Tukey test.. *** $p \leq 0.001$ compared with the control group (treated with saline + vehicle).

64.2%. Pretreatment with L-NAME did not reverse the gastroprotective activity of AFHP (Table 7).

DISCUSSION

Due to the series of side effects associated with the first line of antiulcer drugs, the study of medicinal plants derived compounds for the treatment of various gastrointestinal disorders is becoming important around the world (Zheng et al., 2014). The phytochemical study of *Eugenia uniflora* allowed the isolation and structure elucidation of several phenolic substances of three types: galoil esters, ellagitannins monomeric and dimeric flavonoids and glycosides. Among the compounds identified in the aqueous fraction of hidroacetonic extract of *E. uniflora* leaves are: oenothein B, myricitrin, quercitrin, eugeniflorin D2 and camptothin A (Fortes et al., 2015).

The present study evaluated the gastroprotective effect of aqueous fraction of hydroacetonic leaf extract of pitanga (AFHP) in different models of experimentally induced gastric ulcers and the possible mechanisms of actions involved in this effect. According to Parmar and Desai (1993) various mechanisms in different experimental models of gastric ulcers make it impossible to think of a single mechanism of gastroprotective activity. Recent studies have shown that the leaf extract of *E. dysenterica* and *E. punicifolia* have gastro-protective activity (Basting et al., 2014; Prado et al., 2014).

An important factor involved in the pathogenesis of gastric lesions induced by anti-inflammatory nonsteroidal drugs (NSAIDs) is a deficiency of endogenous prostaglandins (PGs). Prostaglandins, particularly PGE_2 and PGI_2 are described as key mediators in gastric mucosal defense. Its cytoprotective effect has been associated with the stimulation of mucus/bicarbonate secretion, maintenance of mucosal blood flow and inhibition of gastric acid secretion (Martins et al., 2014; Sousa et al., 2013).

The indomethacin (NSAID) inhibits the production of prostaglandins leading to a decrease of the formation and release of mucus/bicarbonate and increases the production of HCl, thereby favoring the appearance of gastrointestinal ulcers (Wallace and Devchand, 2005). Our data suggest that oral treatment with AFHP (100, 300 and 1000 mg/kg), ranitidine (50 mg/kg) protected the gastric mucosal lesions induced by NSAIDs. These results suggest possible involvement of mucus and or PGs.

The stress induced ulcers occur as a result of stressful events such as, burns, sepsis, surgery and trauma (de Almeida et al., 2012). Several studies show that disturbances in gastric secretion, changes in microcirculation and abnormal gastric motility are the possible mechanisms involved in stress-induced ulcers (Amany and Ibrahim, 2013; Batista et al., 2004). Stress reduces endogenous glutathione levels and promotes generation of reactive species such as $OH^{\cdot}$ and inhibit the biosynthesis of mucosal prostaglandins by H_2O_2

accumulation (Bandyopadhyay et al., 2002). The cold restraint stress model (4°C, 2 h) has been widely used to evaluate anti-ulcer activity (Viana et al., 2013). The results suggest that the gastroprotective activity AFHP in stress -induced gastric injury is probably mediated by its antisecretory and antioxidant activity. GSH is a major mediator involved in maintaining the integrity of the gastric mucosa, due to its antioxidant capacity (Mutoh et al., 1990). It is known that gastric lesions induced by stress and EtOH are associated with significant decrease in mucosal levels of GSH (Szabo and Vattay, 1990). The administration of AFHP significantly increased the GSH levels in gastric mucosa. This result suggests that AFHP has antioxidant activity.

The effect of AFHP was also evaluated in ulcer model induced by HCl/EtOH. It is known that the ingestion of large amounts of ethanol promotes gastric lesions in humans and experimental animals (Siegmund et al., 2003; Teyssen and Singer, 2003). It is well established that the formation of gastric mucosal lesions by necrotizing agents such as HCl/EtOH enhances lesions and reduce the number of gastric defense mechanisms such as disruption of blood flow, degranulation of mast cells, reduction of prostaglandin and mucus/bicarbonate release (Abdel-Salam et al., 2001; Glavin and Szabo, 1992). Oral administration of HCl / EtOH in the control group promoted necrotic lesions and bleeding characteristics. Based on the results obtained from this model, AFHP reduced lesion area. An increase in prostaglandin synthesis or mucus adhered to gastric mucosa may be one of the responsible for the antiulcerogenic mechanisms. These results are in agreement with those found by Viana et al. (2013). Mucus is one of the most important parameters that contribute to the protection of gastric mucosa. Gastric mucus is responsible for the first line of defense of the gastric mucosa and consists of a transparent viscous, elastic, adherent gel that is made up of water and glycoproteins (Martins et al., 2015). The mucus layer is a physical barrier that adheres together with bicarbonate and protects the underlying mucosa from proteolytic digestion (Allen and Flemstrom, 2005). When the mucus barrier is damaged, the gastric mucosa becomes more susceptible to gastric acid induced ulcers (Santin et al., 2010). This study revealed that the amount of adhered gastric mucus was increased by treatment with AFHP and this increase probably contributed to the cytoprotective effect of AFHP. Pyloric ligation model produces biochemical parameters in gastric mucosa such as volume, pH and total acidity. The pyloric ligation interferes with gastric mucosa and changes in the levels of prostaglandins, cytokines and endogenous glutathione (Singh et al., 2008).

In an attempt to determine the gastroprotection mechanisms of AFHP, the parameters of gastric acid secretion were evaluated. We found that intraduodenal administration of AFHP significantly reduced total acidity and increased pH. This result indicates a systemic action in addition to anti-secretory activity of AFHP. The reference drug, ranitidine (50 mg/kg), significantly reduced the volume of gastric juice, total acidity and increased the pH.

It is known that nitric oxide (NO) plays an important role in the defense of the gastric mucosa and is a biological mediator which regulates the secretion of mucus and blood flow (Falcao Hde et al., 2013). In the gastrointestinal tract, NO is also involved in modulating the activity of mast cells together with endogenous prostaglandins (Klein-Junior et al., 2013; Wallace, 2006). Our results showed that the inhibition of NO synthase by L-NAME did not reverse gastroprotection effect of AFHP, thereby suggesting that NO synthesis is not critical to its gastroprotective activity.

Conclusion

Overall findings in the current study showed the effectiveness of AFHP in preventing gastric ulcers against lesions induced in various experimental models. These effects could be associated with the gastric cytoprotective mechanisms including the participation of mucus and GSH levels on the mucosa. Further studies will be focused to elucidate the phytochemical(s) responsible(s) for the antiulcer mechanism of AFHP.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

The authors thank Mrs. Ekaterina OPENS Jackson N.L. for the assistance and Prof. Dr. Heleno Ferreira Diaz of the Botany Department, Federal University of Goiás, for the identification of botanical material. We also thank CAPES for the financial support.

REFERENCES

Abdel-Salam OM, Czimmer J, Debreceni A, Szolcsanyi J, Mozsik G (2001). Gastric mucosal integrity: gastric mucosal blood flow and microcirculation. An overview. J. Physiol. Paris 95(1-6):105-127.

Allen A, Flemstrom G (2005). Gastroduodenal mucus bicarbonate barrier: protection against acid and pepsin. Am J. Physiol. Cell Physiol. 288(1):C1-19.

Almeida CE, Karnikowski MG, Foleto R, Baldisserotto B (1995). Analysis of antidiarrhoeic effect of plants used in popular medicine. Rev Saude Publica 29(6):428-433.

Amany N, Ibrahim M (2013). Attenuation of Cold Restraint Stress-Induced Gastric Lesions by Sildenafil in Rats. Med. J. Cairo Univ. 81:229-233.

Amorim AC, Lima CK, Hovell AM, Miranda AL, Rezende CM (2009). Antinociceptive and hypothermic evaluation of the leaf essential oil and isolated terpenoids from *Eugenia uniflora* L. (Brazilian Pitanga).

Phytomedicine 16(10):923-928.
Auricchio M, Bacchi E (2003). *Eugenia uniflora* L. " brazilian cherry" leaves: pharmacobotanical, chemical and pharmacological properties. Rev. Inst. Adolfo Lutz 62(1):55-61.
Bandyopadhyay D, Biswas K, Bhattacharyya M, Reiter RJ, Banerjee RK (2002). Involvement of reactive oxygen species in gastric ulceration: protection by melatonin. Indian J. Exp. Biol. 40(6):693-705.
Basting RT, Nishijima CM, Lopes JA, Santos RC, Lucena Perico L, Laufer S, Bauer S, Costa MF, Santos LC, Rocha LR, Vilegas W, Santos AR, Dos Santos C, Hiruma-Lima CA (2014). Antinociceptive, anti-inflammatory and gastroprotective effects of a hydroalcoholic extract from the leaves of Eugenia punicifolia (Kunth) DC. in rodents. J. Ethnopharmacol. 157:257-267.
Batista LM, de Almeida AB, de Pietro Magri L, Toma W, Calvo TR, Vilegas W, Souza Brito AR (2004). Gastric antiulcer activity of Syngonanthus arthrotrichus SILVEIRA. Biol. Pharm. Bull. 27(3):328-332.
Borrelli F, Izzo AA (2000). The plant kingdom as a source of anti-ulcer remedies. Phytother. Res. 14(8):581-591.
Bouzada MLM, Fabria RL, Duarte GG, Scioa E (2009). Busca de novas drogas antimicrobianas a partir de vegetais.. Juiz de Fora: Principia, Universidade Federal de Juiz de Fora. pp. 1-8
Caldas GF, do Amaral Costa IM, da Silva JB, da Nobrega RF, Rodrigues FF, da Costa JG, Wanderley AG (2011). Antiulcerogenic activity of the essential oil of Hyptis martiusii Benth. (Lamiaceae). J. Ethnopharmacol. 137(1):886-892.
Chaturvedi A, Kumar MM, Bhawani G, Chaturvedi H, Kumar M, Goel RK (2007). Effect of ethanolic extract of Eugenia jambolana seeds on gastric ulceration and secretion in rats. Indian J. Physiol. Pharmacol. 51(2):131-140.
Consolini AE, Sarubbio MG (2002). Pharmacological effects of *Eugenia uniflora* (Myrtaceae) aqueous crude extract on rat's heart. J. Ethnopharmacol. 81(1):57-63.
Corne SJ, Morrissey SM, Woods RJ (1974). Proceedings: A method for the quantitative estimation of gastric barrier mucus. J. Physiol. 242(2):116P-117P.
de Almeida AB, Luiz-Ferreira A, Cola M, Di Pietro Magri L, Batista LM, de Paiva JA, Trigo JR, Souza-Brito AR (2012). Anti-ulcerogenic mechanisms of the sesquiterpene lactone onopordopicrin-enriched fraction from Arctium lappa L. (Asteraceae): role of somatostatin, gastrin, and endogenous sulfhydryls and nitric oxide. J. Med. Food 15(4):378-383.
de Jesus NZ, de Souza Falcao H, Gomes IF, de Almeida Leite TJ, de Morais Lima GR, Barbosa-Filho JM, Tavares JF, da Silva MS, de Athayde-Filho PF, Batista LM (2012). Tannins, peptic ulcers and related mechanisms. Int. J. Mol. Sci. 13(3):3203-3228.
Djahanguiri B (1969). The production of acute gastric ulceration by indomethacin in the rat. Scand. J. Gastroenterol. 4(3):265-267.
Drummond GB, Tom BD (2011a). How can we tell if frogs jump further? Br. J. Pharmacol. 164(2):209-212.
Drummond GB, Tom BD (2011b). Statistics, probability, significance, likelihood: words mean what we define them to mean. Adv. Physiol. Educ. 35(4):361-364.
El-Maraghy SA, Rizk SM, Shahin NN (2015). Gastroprotective effect of crocin in ethanol-induced gastric injury in rats. Chem. Biol. Interact. 229:26-35.
El-Shenawy SM (2009). Evaluation of some pharmacological activities of ethanol extracts of seeds, pericarp and leaves of Eugenia Jamolana in rats. Inflammopharmacology 17(2):85-92.
Falcao Hde S, Maia GL, Bonamin F, Kushima H, Moraes TM, Hiruma Lima CA, Takayama C, Ferreira AL, Souza Brito AR, Agra Mde F, Barbosa Filho JM, Batista LM (2013). Gastroprotective mechanisms of the chloroform and ethyl acetate phases of Praxelis clematidea (Griseb.) R.M.King & H.Robinson (Asteraceae). J. Nat. Med. 67(3):480-491.
Ferro E, Schinini A, Maldonado M, Rosner J, Hirschmann GS (1988). *Eugenia uniflora* leaf extract and lipid metabolism in Cebus apella monkeys. J. Ethnopharmacol. 24(2-3):321-325.
Fortes GAC, Carvalho AG, Ramalho RRF, Silva AJR , Ferri PH, Santos SC (2015). Antioxidant activities of hydrolysable tannins and flavonoid glycosides isolated from *Eugenia uniflora* L. Rec. Nat. Prod. 9(2):251-256.
Glavin GB, Szabo S (1992). Experimental Gastric-Mucosal Injury - Laboratory Models Reveal Mechanisms of Pathogenesis and New Therapeutic Strategies. Faseb J. 6(3):825-831.
Irwin S (1968). Comprehensive observational assessment: Ia. A systematic, quantitative procedure for assessing the behavioral and physiologic state of the mouse. Psychopharmacologia 13(3):222-257.
Ishikawa T, Donatini RDS, Diaz IEC, Yoshida M, Bacchi EM, Kato ETM (2008). Evaluation of gastroprotective activity of Plinia edulis (Vell.) Sobral (Myrtaceae) leaves in rats. J. Ethnopharmacol. 118(3):527-529.
Klein-Junior LC, Santin JR, Lemos M, Silveira AC, Rocha JA, Beber AP, Wagner TM, Bresolin TM, Bella-Cruz A, Cechinel-Filho V, Faloni de Andrade S (2013). Role of gastric mucus secretion, oxinitrergic system and sulfhydryl groups on the gastroprotection elicited by Polygala cyparissias (Polygalaceae) in mice. J. Pharm. Pharmacol. 65(5):767-776.
Lima VLAG, Melo EA, Des L (2002). Total phenolics and carotenoids in surinam cherry. Sci. Agric. 59(3):447-450.
Lopes M (2008). Composição química, atividade antibacteriana e alelopática dos óleos essenciais de *Eugenia uniflora* L. E Myrciaria glazioviana (kiaersk) G. M. Barroso & Sobral (Myrtaceae)Universidade Federal de Viçosa, Viçosa.
Lorenzi H, Souza HM (1999). Plantas ornamentais do Brasil. Pages 1088 in Odessa N, ed. São Paulo: Instituto Plantarum.
Markman BE, Bacchi EM, Kato ET (2004). Antiulcerogenic effects of Campomanesia xanthocarpa. J. Ethnopharmacol. 94(1):55-57.
Martins JL, Rodrigues OR, de Sousa FB, Fajemiroye JO, Galdino PM, Florentino IF, Costa EA (2015). Medicinal species with gastroprotective activity found in the Brazilian Cerrado. Fundam. Clin. Pharmacol. 29(3):238-251.
Martins JL, Rodrigues OR, da Silva DM, Galdino PM, de Paula JR, Romao W, da Costa HB, Vaz BG, Ghedini PC, Costa EA (2014). Mechanisms involved in the gastroprotective activity of Celtis iguanaea (Jacq.) Sargent on gastric lesions in mice. J. Ethnopharmacol. 155(3):1616-1624.
Matsuda H, Li Y, Yoshikawa M (1999). Roles of capsaicin-sensitive sensory nerves, endogenous nitric oxide, sulfhydryls, and prostaglandins in gastroprotection by momordin Ic, an oleanolic acid oligoglycoside, on ethanol-induced gastric mucosal lesions in rats. Life Sci. 65(2):27-32.
Mutoh H, Hiraishi H, Ota S, Ivey KJ, Terano A, Sugimoto T (1990). Role of oxygen radicals in ethanol-induced damage to cultured gastric mucosal cells. Am. J. Physiol. 258(4 Pt 1):G603-609.
Parmar NS, Desai JK (1993). A review of the current methodology for the evaluation of gastric and duodenal anti-ulcer agents. Ind. J. Pharmacol. 25:120-135.
Prado LC, Silva DB, de Oliveira-Silva GL, Hiraki KR, Canabrava HA, Bispo-da-Silva LB (2014). The gastroprotective effects of Eugenia dysenterica (Myrtaceae) leaf extract: the possible role of condensed tannins. Biol. Pharm. Bull. 37(5):722-730.
Rattmann YD, de Souza LM, Malquevicz-Paiva SM, Dartora N, Sassaki GL, Gorin PAJ, Iacomini M (2012). Analysis of Flavonoids from *Eugenia uniflora* Leaves and Its Protective Effect against Murine Sepsis. Evid-Based Complement. Altern. Med. 1:1-9.
Rios ER, Rocha NF, Venancio ET, Moura BA, Feitosa ML, Cerqueira GS, Soares PM, Woods DJ, de Sousa FC, Leal LK, Fonteles MM (2010). Mechanisms involved in the gastroprotective activity of esculin on acute gastric lesions in mice. Chem. Biol. Interact. 188(1):246-254.
Santin JR, Lemos M, Klein Junior LC, Niero R, de Andrade SF (2010). Antiulcer effects of Achyrocline satureoides (Lam.) DC (Asteraceae) (Marcela), a folk medicine plant, in different experimental models. J. Ethnopharmacol. 130(2):334-339.
Santos KK, Matias EF, Tintino SR, Souza CE, Braga MF, Guedes GM, Costa JG, Menezes IR, Coutinho HD (2013). Enhancement of the antifungal activity of antimicrobial drugs by *Eugenia uniflora* L. J. Med. Food 16(7):669-671.
Santos KK, Matias EF, Tintino SR, Souza CE, Braga MF, Guedes GM, Rolon M, Vega C, de Arias AR, Costa JG, Menezes IR, Coutinho HD (2012). Anti-Trypanosoma cruzi and cytotoxic activities of *Eugenia uniflora* L. Exp. Parasitol. 131(1):130-132.
Schapoval EE, Silveira SM, Miranda ML, Alice CB, Henriques AT

(1994). Evaluation of some pharmacological activities of *Eugenia uniflora* L. J. Ethnopharmacol. 44(3):137-142.
Sedlak J, Lindsay RH (1968). Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal. Biochem. 25(1):192-205.
Senay EC, Levine RJ (1967). Synergism between cold and restraint for rapid production of stress ulcers in rats. Proceed. Soc. Exp. Biol. Med. 124(4):1221-1224.
Shay H, Komarov SA, Fels SS, Meranze D, Gruenstein M, Siplet H (1945). A simple method for the uniform production of gastric ulceration in the rat. Gastroenterology 5(1):43-61.
Siegmund S, Haas S, Schneider A, Singer MV (2003). Animal models in gastrointestinal alcohol research - a short appraisal of the different models and their results. Best Pract. Res. Clin. Gastroenterol. 17(4):519-542.
Singh S, Khajuria A, Taneja SC, Khajuria RK, Singh J, Johri RK, Qazi GN (2008). The gastric ulcer protective effect of boswellic acids, a leukotriene inhibitor from Boswellia serrata, in rats. Phytomedicine 15(6-7):408-415.
Sousa FB, Martins JL, Florentino IF, Couto RO, Nascimento MV, Galdino PM, Ghedini PC, Paula JR, Costa EA (2013). Preliminary studies of gastroprotective effect of *Celtis iguanaea* (Jacq.) Sargent leaves (Ulmaceae). Nat. Prod. Res. 27(12):1102-1107.
Sumbul S, Ahmad MA, Asif M, Saud I, Akhtar M (2010). Evaluation of *Myrtus communis* Linn. berries (common myrtle) in experimental ulcer models in rats. Hum. Exp. Toxicol. 29(11):935-944.
Szabo S, Vattay P (1990). Experimental gastric and duodenal ulcers. Advances in pathogenesis. Gastroenterol. Clin. North Am. 19(1):67-85.
Teyssen S, Singer MV (2003). Alcohol-related diseases of the oesophagus and stomach. Best Pract. Res. Clin. Gastroenterol. 17(4):557-573.
Viana AF, Fernandes HB, Silva FV, Oliveira IS, Freitas FF, Machado FD, Costa CL, Arcanjo DD, Chaves MH, Oliveira FA, Oliveira RC (2013). Gastroprotective activity of *Cenostigma macrophyllum* Tul. var. acuminata Teles Freire leaves on experimental ulcer models. J. Ethnopharmacol. 150(1):316-323.
Victoria FN, de Siqueira Brahm A, Savegnago L, Lenardao EJ (2013). Involvement of serotoninergic and adrenergic systems on the antidepressant-like effect of *E. uniflora* L. leaves essential oil and further analysis of its antioxidant activity. Neurosci. Lett. 544:105-109.
Victoria FN, Lenardao EJ, Savegnago L, Perin G, Jacob RG, Alves D, da Silva WP, da Motta Ade S, Nascente Pda S (2012). Essential oil of the leaves of *Eugenia uniflora* L.: antioxidant and antimicrobial properties. Food Chem. Toxicol. 50(8):2668-2674.
Wallace JL (2006). Nitric oxide, aspirin-triggered lipoxins and NO-aspirin in gastric protection. Inflamm. Allergy Drug Targets 5(2):133-137.
Wallace JL, Devchand PR (2005). Emerging roles for cyclooxygenase-2 in gastrointestinal mucosal defense. Br. J. Pharmacol. 145(3):275-282.
Weyerstahl P, Marschall-Weyerstahl H, Christiansen C, Oguntimein BO, Adeoye AO (1988). Volatile constituents of *Eugenia uniflora* leaf oil. Planta Med. 54(6):546-549.
Zanatta F, Gandolfi RB, Lemos M, Ticona JC, Gimenez A, Clasen BK, Cechinel Filho V, de Andrade SF (2009). Gastroprotective activity of alkaloid extract and 2-phenylquinoline obtained from the bark of Galipea longiflora Krause (Rutaceae). Chem. Biol. Interact. 180(2):312-317.
Zheng YF, Xie JH, Xu YF, Liang YZ, Mo ZZ, Jiang WW, Chen XY, Liu YH, Yu XD, Huang P, Su ZR (2014). Gastroprotective effect and mechanism of patchouli alcohol against ethanol, indomethacin and stress-induced ulcer in rats. Chem. Biol. Interact. 222:27-36.

Supplementary Table 1.

Route of administration	Dose (mg/kg)	Observed changes
Intraperitoneal	100	Reduction of spontaneous movement (up until 4 hrs), analgesia and alienation (5, 10, 20, 30 and 60 min)
	300	Reduction of spontaneous movement (up to 4 h), catatonia, analgesia, ataxia, alienation, diarrhea, contortion (up to 4 h) and death within 24 h
	1000	Reduction of spontaneous movement (up to 4 h), catatonia, analgesia, ataxia, alienation, diarrhea, contortion (up to 4 h) and death within 24 h
Oral		No changes observed.