**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**

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*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 model 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 uniflora* (Kunth) DC. (Basting et al., 2014) among others, have gastroprotective activity.

*Eugenia uniflora* L. (Myrtaceae) is popularly known as cherry, ibiipitanga, pitanga or naganpuri (Consolini and Sarubbo, 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 American 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 (Schipoval et al., 1994), antispasmodic (Amorim et al., 2009), antihypertensive (Consolini and Sarubbo, 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 hydroacetonite leaf extract of the pitanga (AFHP).

**MATERIALS AND METHODS**

**Botanical material**

The *E. uniflora* leaves were collected at Anápolis, Goiás, Brazil (16°20' 12.8 "S, 48°56' 19.3 "W). The botanical material was authenticated by Prof. Dr. Heleno Ferreira Díaz of the Botany Department, Federal University of Goiás. A specimen voucher was deposited at the herbarium of the Federal University of Goiás (nº 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 hydroacetonite 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), carbonoxolone, L-Name (NG-nitro-L-arginine), indomethacin and Ailcin 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 (Vetc, Duque de Caxias, RJ, Brazil), sodium hydroxide (Lafan, Varzea Paulista, SP, Brazil), Ethylenediaminetetraacetic acid (EDTA), 5,5-ditiole-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 freeambulation 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).
**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 remove 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 ligation 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 ligation 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 80 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 MgCl₂ (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 agitation 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 Sedik 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 μL of 5-thiobis-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.

<table>
<thead>
<tr>
<th>Index of lesion</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Discoloration of mucosa</td>
<td>1</td>
</tr>
<tr>
<td>Edema</td>
<td>1</td>
</tr>
<tr>
<td>Hemorrhages</td>
<td>1</td>
</tr>
<tr>
<td><strong>Number of petechia</strong></td>
<td></td>
</tr>
<tr>
<td>Until 25%</td>
<td>2</td>
</tr>
<tr>
<td>More than 25%</td>
<td>3</td>
</tr>
<tr>
<td><strong>Intensity of ulceration</strong></td>
<td></td>
</tr>
<tr>
<td>Ulcers or erosion up to 1 mm</td>
<td>N × 2</td>
</tr>
<tr>
<td>Ulcers or erosion larger than 1 mm</td>
<td>N × 3</td>
</tr>
<tr>
<td>Perforated ulcers</td>
<td>N × 4</td>
</tr>
</tbody>
</table>

N, Number of stomach lesions.

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

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

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.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment (p.o.)</th>
<th>Dose (mg/kg)</th>
<th>Index of lesion</th>
<th>Reduction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Vehicle</td>
<td>-</td>
<td>9.1±1.0</td>
<td>-</td>
</tr>
<tr>
<td>Ranitidine</td>
<td>Ranitidine</td>
<td>50</td>
<td>5.1±0.3***</td>
<td>43.8</td>
</tr>
<tr>
<td>AFHP</td>
<td>AFHP</td>
<td>300</td>
<td>5.1±0.2***</td>
<td>43.8</td>
</tr>
</tbody>
</table>

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.

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.

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

Results are expressed as mean ± SEM of the ulcerated area (%) for eight mice. Statistical comparison was performed using ANOVA followed by the Tukey. *** p ≤ 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.

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

Results are expressed as mean ± SEM for eight mice. Statistical comparison was performed using ANOVA followed by the Tukey. * p ≤ 0.05, ** p ≤ 0.01, *** p ≤ 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.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment (p.o.)</th>
<th>Dose (mg/kg)</th>
<th>Alcian blue (µg/g tissue)</th>
<th>GSH (µg/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control without lesion</td>
<td>Vehicle + water</td>
<td>-</td>
<td>38.4±2.3</td>
<td>150.4±5.6</td>
</tr>
<tr>
<td>Control with lesion</td>
<td>Vehicle + EtOH</td>
<td>-</td>
<td>29.1±2.1*</td>
<td>128.0±3.0**</td>
</tr>
<tr>
<td>Carbenoxolone</td>
<td>Carbenoxolone + EtOH</td>
<td>200</td>
<td>41.0±1.4##</td>
<td>143.5±3.8#</td>
</tr>
<tr>
<td>AFHP</td>
<td>AFHP + EtOH</td>
<td>300</td>
<td>37.5±1.7#</td>
<td>190.2±11.8##</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM for eight mice. Statistical comparison was performed using ANOVA followed by the Tukey test. * p ≤ 0.05, ** p ≤ 0.01, control without lesion vs control with lesion; # p ≤ 0.05, ## p ≤ 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.

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

Results are expressed as mean ± SEM for eight mice. Statistical comparison was performed using ANOVA followed by the Tukey test. *** p ≤ 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: galol esters, ellagitannins monomeric and dimeric flavonoids and glycosides. Among the compounds identified in the aqueous fraction of hydroacetic 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 hydroacetic 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 PGE2 and PG12 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· and inhibit the biosynthesis of mucosal prostaglandins by H2O2.
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.

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REFERENCES


Scapoval EE, Silveira SM, Miranda ML, Alice CB, Henriques AT...


### Supplementary Table 1.

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