• Research Article

Gastric antisecretory and cytoprotective effects of hydroalcoholic extracts of *Plumeria alba* Linn. leaves in rats

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OBJECTIVE: This study was conducted to evaluate the antiulcerogenic property of hydroalcoholic extract obtained from the leaves of *Plumeria alba* Linn.

METHODS: Antulcer assays were performed using the protocols of ulcer induced by non-steroidal anti-inflammatory drugs, ethanol and pylorus ligation. The hydroalcoholic extract (HAPA), and various fractions of HAPA like, n-hexane extract (HPA), ethyl acetate extract (EAPA) and n-butanol extract (BPA) were administered at doses of 200 and 400 mg/kg for HAPA and 100 and 200 mg/kg for fractions of extracts. Parameters of gastric secretion (volume, pH, total protein, and free and total acidity) were determined by the pylorus ligation model. Parameters like aspartate aminotransferase and alanine aminotransferase were also determined in ethanol-induced ulcer model. To determine the mechanism of action, role of nitric oxide was also evaluated.

RESULTS: EAPA and BPA (100 and 200 mg/kg, p.o.) showed gastric ulcer-healing effect in indomethacin-induced ulcer model, while HAPA (200 mg/kg) and HPA showed no significant antiulcer effect. Both EAPA and BPA showed gastric cytoprotective effect in ethanol-induced gastric ulcer and inhibited gastric secretion in pylorus-ligated rats.

CONCLUSION: The results of the present study show that some hydroalcoholic extract of *Plumeria alba* L. displays antiulcer activity, as demonstrated by the significant inhibition of ulcer formation induced by different models, which is consistent with the literature report in folk medicine.

KEYWORDS: *Plumeria*; anti-ulcer agents; plant extracts; rats

http://dx.doi.org/10.1016/S2095-4964(14)60002-9
Received August 13, 2013; accepted October 15, 2013.
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1 Introduction

Gastric ulcer is a chronic disease that affects millions of people worldwide which involves disruption in the skin or mucus membrane lining alimentary canal[2,3]. Ulceration occurs when there is imbalance between aggressive (acid-pepsin secretions) and protective factors (such as mucus secretion, mucosal barrier, cell regeneration, blood flow and prostaglandins)[2,3]. Various factors such as smoking, poor diet, alcohol, stress, infections (*Helicobacter pylori*) and frequent or indiscriminate use of non-steroidal anti-inflammatory drugs (NSAIDs), have been implicated in the aetiology of gastric ulcer[4]. There are two main approaches for treatment of peptic ulcer. The first is to reduce gastric acid production and the second is to enhance gastric mucosa protection[5,6]. The current treatment of peptic ulcer is mainly performed with H₂ receptor antagonists,
antacids, proton pump inhibitors, and antimuscarinics. But, prolonged use of these treatments produces adverse reactions like, hypersensitivity, arrhythmia, impotence, gynecomasia and hematopoietic disorders[74]. Therefore, there is a strong need for new effective drugs for peptic ulcer therapy[9]. In this regard, many plant species used in traditional and folk medicine for the relief of gastric symptoms have been studied to establish their pharmacological activity[10]. Several medicinal plants have been proven to be safe and effective with better patient tolerance. They are shown to be less expensive, and therefore globally competitive. These plants among others are currently being screened for their gastroprotective activity in animal studies[11-13].

Genus Plumeria (Apocynaceae) consists of some flowering trees which originated from Central America and its different species are now distributed in the warmer regions of the world including India. The genus is represented by topical trees or shrubs, frequently cultivated as ornamental and medicinal plants[14]. The bark, root, leaves and flowers are used for medicinal purposes. Some of the medicinally important species are P. accuminata, P. alba, P. rubra, P. lancifolia, P. drastic, P. phagidenica, etc. In traditional system of medicine, the plants of Plumeria species are widely used as a purgative, rubefacient in rheumatism, asthma, piles, gonorrhea, blood disorders and tumors[15].

Plumeria alba Linn. is commonly known as White Champa cultivated in Indian gardens. The milky sap of the stem and leaf is applied to skin diseases such as herpes, scabies and ulcers[16]. Moreover its bark is bruised and applied as plaster over hard tumors[17]. Whereas the latter taxon finds use as purgative, cardiotonic, diuretic and hypotensive[18]. The plant is reported to contain amyrin acetate, mixture of amyrins, β-sitosterol, scopotetin, iridoids isoplumericin, plumieride coumerate and plumieride coumerate glucoside[19,20].

As far as we are aware, there have been no reports on the gastroprotective effect of this plant in animals so far. In this study, hydroalcoholic extract and fractions of P. alba were evaluated in animals to substantiate and expand its clinical applications.

2 Materials and methods

2.1 Plant materials

The leaves of P. alba were collected from Chaudhary Devi Lal Park in February (termination of winter), 2011 in Yamunanagar, in the state of Haryana, India and identified by Dr. H.B. Singh, Head, Raw Materials Herbarium and Museum (RHMD), New Delhi, India. A voucher specimen of the plant (Ref. No. NISCAIR/RHMD/CONSULT/2012-13/2047/55) has been preserved there for future references.

2.2 Drugs, reagents and solvents

Indomethacin, ranitidine, L-arginine, L-nitro arginine methyl ester (L-NAME) (Sigma-Aldrich, USA) and the solvents used for extraction and chemicals used for phytochemical analysis were of analytical grade and procured from local firms.

2.3 Extraction

The air-dried leaves (2 kg) were thoroughly washed under running tap water so as to remove any type of contamination and air-dried in shade, powdered in grinder and passed through sieve of mesh size no-40. The extraction was done with aqueous ethanol (30:70) mixture by hot Soxhlet extraction method and the extract was concentrated in a rotary evaporator under reduced pressure, yielding 720 g (36%) of crude hydroalcoholic extract (HAPA). The dried crude extract was preserved in airtight glass container at 4-8 °C. With acute model of indomethacin-induced ulcer, HAPA was found very active and it was then fractionated with solvents of increasing polarity. Initially, HAPA was partition-fractionated with 1:1 (volume ratio) of n-hexane and ethanol (50%), and the mixture was shaken vigorously and kept for about 30 min to make the two layers separate. The upper layer consisting of n-hexane was removed and concentrated in a rotary evaporator to obtain n-hexane extract (HPA). The same procedure was repeated with the bottom layer by using equivalent volume of other solvents like, dichloromethane, ethyl acetate, n-butanol, and ethanol to obtain DCMPA, EAPA, BPA, and EPA, respectively.

2.4 Preliminary phytochemical analysis

Phytochemical analysis was performed using standard procedures to identify chemical constituents as described by Khandelwal[21]. Thin layer chromatography was used to evaluate the chemical composition of HAPA, HPA, EAPA and BPA extracts. The plates were sprayed with natural products reagent and polyethylene glycol (NP/PEG) for detecting flavonoids. Phenolic compounds are detected after exposing the plates to ammonia vapours and fluorescent spots were immediately observed under ultraviolet light. Total phenols were detected with 5% ferric chloride solution in methanol[22].

2.5 Animals

Healthy Wistar rats (175-250 g) of either sex were obtained from a disease-free animal house of Chaudhary Charan Singh, Haryana Agriculture University, Hisar, Haryana (India). The animals were housed in the animal house, Institute of Pharmaceutical Sciences, Kurukshetra University, Kurukshetra, Haryana (India). Rats were fed with commercially available food and were maintained under standard conditions of temperature ((25±5) °C), relative humidity (55%-±10%), and 12/12 h light/dark cycle. They were transferred to the laboratory 12 h prior to the experiments and given only water ad libitum. In all the
experiments, the animals were kept in cages with raised floors of wide mesh, to prevent coprophagy. The animals were housed and cared in accordance with the federal government legislation on animal care. The experiments were also authorized by the Institutional Animals Ethical Committee for Animal Care (Register Number: 562/GO/02/a/CPCSEA) and were in accordance with the Committee for the Purpose of Control and Supervision of Experiments on Animals Guidelines, Government of India.

2.6 Evaluation of antiulcer activity

2.6.1 Doses and route of administration

For experimentation doses of 200 and 400 mg/kg HAPA and 100 and 200 mg/kg various fractions of HAPA were used. Fresh drug solutions were prepared in sterile distilled water at the time of administration and were administered per oral (p.o.) so as to avoid any additional stress to the animals.

2.6.2 NSAID-induced ulcer

The experiment was performed according to the method described by Djahanguiri,[23] with a few modifications. After 24 h of fasting, the rats were randomly divided into ten groups of six animals each. The rats were treated with different leaf extracts of *P. alba* or ranitidine (50 mg/kg), while control group was given 0.5 mL of vehicle (1% Tween-80 aqueous solution). All the treatments were administered orally. One hour after treatment, all the rats received indomethacin (20 mg/kg) to induce gastric ulcer. Four hours after treatment with indomethacin, the animals were sacrificed by cervical dislocation. The stomachs were removed and opened along the greater curvature, then gently rinsed with water to remove the gastric contents and blood clots, for subsequent scanning. The images obtained were analyzed using the parameters described in the section on stomach analysis.

2.6.3 Ethanol-induced gastric mucosal lesions

The experiment was performed according to the method described by Mizui and Doteuchi[24] with slight modifications. After 36 h of fasting, the rats were randomly divided into four groups of six animals each. The first group was given 0.5 mL of vehicle (1% Tween-80 aqueous solution), and the second and third groups were given 100 mg/kg EAPA and 200 mg/kg BPA, respectively. The fourth group received ranitidine (50 mg/kg). All the samples were administered per oral (p.o.) so as to avoid any additional stress to the animals. After 30 min all the animals received ethanol. After 30 min of treatments, all the groups of animals received L-NAME (70 mg/kg, i.p.) and L-arginine (200 mg/kg, i.p.) were administered to animals. After 30 min all the animals received L-NAME (70 mg/kg, i.p.) and L-arginine (200 mg/kg, i.p.) were administered to animals. After 30 min of treatments, all the groups of animals received L-NAME (70 mg/kg, i.p.) and L-arginine (200 mg/kg, i.p.) were administered to animals. After 30 min of treatments, all the groups of animals received L-NAME (70 mg/kg, i.p.) and L-arginine (200 mg/kg, i.p.) were administered to animals. After 30 min of treatments, all the groups of animals received L-NAME (70 mg/kg, i.p.) and L-arginine (200 mg/kg, i.p.) were administered to animals. After 30 min of treatments, all the groups of animals received L-NAME (70 mg/kg, i.p.) and L-arginine (200 mg/kg, i.p.) were administered to animals.

2.6.4 Gastric acid secretion

The determination of gastric secretion was performed using the method of Shay *et al*[25], with a few modifications. The rats were divided into four groups (n = 6). After 24 h of fasting, the animals were anesthetized, and the abdomen was incised and the pylorus ligated. Immediately after the pylorus ligation, EAPA and BPA were administered at doses of 100 and 200 mg/kg respectively. Ranitidine (50 mg/kg) was used as a positive control, and 0.5 mL of vehicle (1% Tween-80 aqueous solution) was administered as a negative control. All the samples were administered intraduodenally. Four hours later, the animals were sacrificed by cervical dislocation; the abdomen was opened, and another ligature was placed around the oesophagus close to the diaphragm. The stomachs were removed and the gastric content was collected and drained into a graduated centrifuge tube. The pH and volume of gastric juice were measured after centrifugation at 2 000 × g for 10 min. From the supernatant, aliquots were taken for the determination of total and free acidity.

2.6.5 Ethanol-induced ulcer in rats pretreated with L-NAME

A modified method of Matsuda *et al*[26] was used to study the role of endogenous nitric oxide (NO) in gastro-protective effects of the extracts. The first group was given 0.5 mL of vehicle (1% Tween-80 aqueous solution), then EAPA and BPA (100 and 200 mg/kg, p.o.) respectively and L-arginine (200 mg/kg, i.p.) were administered to animals. After 30 min of treatments, all the groups of animals received L-NAME (70 mg/kg, i.p.) and L-arginine (200 mg/kg, i.p.) were administered to animals. After 30 min of treatments, all the groups of animals received L-NAME (70 mg/kg, i.p.) and L-arginine (200 mg/kg, i.p.) were administered to animals. After 30 min of treatments, all the groups of animals received L-NAME (70 mg/kg, i.p.) and L-arginine (200 mg/kg, i.p.) were administered to animals.

2.7 Stomach analysis

2.7.1 Percentage of inhibition

The percentage of inhibition was calculated using the following formula:

\[
I(\%) = \left(\frac{UI_{control} - UI_{test}}{UI_{control}}\right) \times 100
\]

Where *I* = inhibition, *UI* = ulcer index.

2.7.2 Ulcer index

The mucosal layer of the stomach was observed under a magnifying lens and ulcers were checked. The area (mm²) of all lesions was measured using digital callipers to give a gastric damage score. The ulcer index was determined using the following formula[27]:

\[
UI = \frac{10}{X}
\]

Where *X* = total mucosal area/total ulcerated area.

2.7.3 Total acidity and free acidity determination

About 1 mL of centrifuged and filtered gastric juice was taken in a conical flask. Two drops of 1% phenolphthalein indicator for total acidity and Topfer’s reagent for free
acidity were added to it. It was titrated against 0.1 mol/L sodium hydroxide until a permanent pink color (total acidity) or canary yellow color (free acidity) was observed. The total/free acidity is expressed as mmol/h by the following formula:

\[
\text{Total/free acidity} = n \times 0.01 \times 36.45 \times 1000
\]

Where, \( n \) is the volume of sodium hydroxide (NaOH) consumed, 0.01 is normality of NaOH, 36.45 is molecular weight of NaOH, and 1000 is the factor (to be represented in litre).

2.7.4 Biochemical parameters

Blood samples were analyzed for aspartate aminotransferase (AST) and alanine aminotransferase (ALT) level estimation in ethanol-induced gastric lesions and gastric juice total protein was calculated in pylorus ligation method.

2.8 Histological analysis

After sacrifice of animals, stomach tissues were removed from the body, and fixed for 24 h in 10% formalin. After decalcification in 5% formic acid, paraffin-embedded tissue sections (7 µm thick) were stained with hematoxylin and eosin. An experienced pathologist (Dr. Neeraj Mittal), unaware of the different drug treatments, evaluated the slides under a light microscope for the presence of congestion, hemorrhage, edema, necrosis, inflammatory and ulcerations.

2.9 Statistical analysis

All the values were expressed as mean ± standard error of mean. The statistical significance of difference among groups was analyzed using one-way analysis of variance, followed by Dunnett’s \( t \) test and Turkey-Kramer multiple comparison test. A value of \( P < 0.05 \) was considered significant using Graph-Pad Prism version 5.04 for Windows (Graph Pad Software, San Diego, California, USA).

3 Results

3.1 Preliminary phytochemical screening

The percentage yield of HAPA, HPA, EAPA and BPA was found to be 36.0%, 6.1%, 18.2% and 16.5% (all in weight ratio), respectively. Preliminary phytochemical screening of leaf extract revealed the presence of steroids, alkaloids, tannins, glycosides, carbohydrates, saponins and flavonoids in HAPA; both EAPA and BPA contained alkaloids, glycosides, flavonoids, saponins and steroids. In addition, tannins were also present in BPA; HPA showed the presence of carbohydrates only (Table 1).

3.2 Antiulcer activity

3.2.1 Indomethacin-induced gastric ulcer

Oral administration of indomethacin (20 mg/kg) produces gastric mucosal ulceration in the rats, extensively in the control group. As shown in Table 2, pretreatment with HAPA, HPA EAPA and BPA (100 and 200 mg/kg) produced significant inhibition reaching 28.8%, 51.8%, 28.1%, 28.1%, 58.5%, 59.2%, 45.9% and 60.0%, respectively. Ranitidine (50 mg/kg) showed 62.2% of ulcer inhibition. This experimental model provides the screening of doses and efficacy of the treatments against ulcer formation. Results showed that EAPA and BPA provided significant gastro-protection at both the tested doses (\( P < 0.01 \)), while HAPA only produced good protection at dose of 400 mg/kg (\( P < 0.01 \)). Thereby we focused the following protocols using the minor doses capable to produce the more effective gastro-protection, which were EAPA 100 mg/kg and BPA 200 mg/kg.

3.2.2 Ethanol-induced gastric ulcer

Oral administration of 95% ethanol induced characteristic lesions (haemorrhagic streaks in glandular gastric mucosa), extensively in the vehicle control group. Pretreatment with EAPA and BPA (100 and 200 mg/kg), respectively reduced ulcerated area significantly (\( P < 0.01 \)), with inhibition percentage of 62.22% and 55.55%, respectively. Standard drug ranitidine (50 mg/kg) also significantly (\( P < 0.01 \)) inhibited the gastric injury by 75.55% (Table 3 and Figures 1 and 2).

3.2.3 Pylorus ligation-induced gastric ulcer

The interference of the extracts on the parameters of gastric secretion was also evaluated using the pylorus ligation method. Following the administration of EAPA

<table>
<thead>
<tr>
<th>Phytoconstituents</th>
<th>Hydroalcoholic extract</th>
<th>n-Hexane extract</th>
<th>Ethyl acetate extract</th>
<th>n-Butanol extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>++</td>
<td>-</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
<td>-</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+</td>
<td>++</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+: slightly present; ++: moderately present; +++: highly present; -: not detected.
Table 2  Ulcer index of indomethacin-induced gastric ulcers

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Ulcer index</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indomethacin (20 mg/kg, p.o.)</td>
<td>6</td>
<td>1.35±0.15</td>
<td>0</td>
</tr>
<tr>
<td>HAPA (200 mg/kg, p.o.)</td>
<td>6</td>
<td>0.96±0.08**</td>
<td>28.8</td>
</tr>
<tr>
<td>HAPA (400 mg/kg, p.o.)</td>
<td>6</td>
<td>0.65±0.02**</td>
<td>51.8</td>
</tr>
<tr>
<td>HPA (100 mg/kg, p.o.)</td>
<td>6</td>
<td>0.97±0.06</td>
<td>28.1</td>
</tr>
<tr>
<td>HPA (200 mg/kg, p.o.)</td>
<td>6</td>
<td>0.97±0.12</td>
<td>28.1</td>
</tr>
<tr>
<td>EAPA (100 mg/kg, p.o.)</td>
<td>6</td>
<td>0.56±0.03**</td>
<td>58.5</td>
</tr>
<tr>
<td>EAPA (200 mg/kg, p.o.)</td>
<td>6</td>
<td>0.55±0.02**</td>
<td>59.2</td>
</tr>
<tr>
<td>BPA (100 mg/kg, p.o.)</td>
<td>6</td>
<td>0.73±0.11**</td>
<td>45.9</td>
</tr>
<tr>
<td>BPA (200 mg/kg, p.o.)</td>
<td>6</td>
<td>0.54±0.03**</td>
<td>60.0</td>
</tr>
<tr>
<td>Ranitidine (50 mg/kg, p.o.)</td>
<td>6</td>
<td>0.51±0.03**</td>
<td>62.2</td>
</tr>
</tbody>
</table>

Ulcer index is expressed as mean ± standard error of mean. *P < 0.05, **P < 0.01, vs indomethacin group.

HAPA: hydroalcoholic extract; HPA: n-hexane extract; EAPA: ethyl acetate extract; BPA: n-butanol extract.

Table 3  Ulcer index of alcohol-induced gastric ulcers

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Ulcer index</th>
<th>Inhibition (%)</th>
<th>ALT (IU/L)</th>
<th>AST (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol (1 mL, p.o.)</td>
<td>6</td>
<td>0.90±0.01</td>
<td>0</td>
<td>65.98±2.50</td>
<td>353.00±2.10</td>
</tr>
<tr>
<td>EAPA (100 mg/kg, p.o.)</td>
<td>6</td>
<td>0.34±0.03 △△</td>
<td>62.2</td>
<td>54.83±2.04 △△</td>
<td>307.50±3.81 △△</td>
</tr>
<tr>
<td>BPA (200 mg/kg, p.o.)</td>
<td>6</td>
<td>0.40±0.05 △△</td>
<td>55.55</td>
<td>48.35±1.77 △△</td>
<td>323.33±8.13 △△</td>
</tr>
<tr>
<td>Ranitidine (50 mg/kg, p.o.)</td>
<td>6</td>
<td>0.22±0.02 △△</td>
<td>75.55</td>
<td>55.18±3.50 △△</td>
<td>331.30±3.07 △△</td>
</tr>
</tbody>
</table>

Data of ulcer index, ALT and AST are expressed as mean ± standard error of mean. △ P < 0.05, △△ P < 0.01, vs alcohol group.

ALT: alanine aminotransferase; AST: aspartate aminotransferase; EAPA: ethyl acetate extract; BPA: n-butanol extract.

Figure 1  Effects of EAPA and BPA on macroscopic changes in ethanol-induced gastric ulcers

Results showed that the rats pretreated with EAPA and BPA at doses of 100 and 200 mg/kg had considerably reduced areas of gastric ulcer formation compared to rats pretreated with only Tween-80 (ulcer control). A: Alcohol control group; B: EAPA group; C: BPA group; D: Ranitidine group.

EAPA: ethyl acetate extract; BPA: n-butanol extract.

Figure 2  Histological slices of the stomach tissues stained by hematoxylin and eosin from rats submitted to ethanol-induced gastric ulcer assay (Light microscopy, × 100)

A: Control group damaged by ethanol; B: EAPA group treated with ethanol after pretreatment with EAPA; C: BPA group treated with ethanol after pretreatment with BPA; D: Ranitidine group treated with ethanol after pretreatment with ranitidine.

EAPA: ethyl acetate extract; BPA: n-butanol extract.
and BPA it was observed that the extracts and positive control (ranitidine) showed significant ($P < 0.05$, $P < 0.01$) reduction in ulcer index, total acidity, free acidity, volume of gastric juice and elevation of gastric pH. None of the treatment except ranitidine produced a significant ($P < 0.01$) increase in total protein (Table 4 and Figures 3 and 4). These results suggest that the extracts interfered with gastric secretion.

### 3.2.4 Ethanol-induced ulcer in rats pretreated with L-NAME

The results obtained for the gastroprotective effect of EAPA and BPA, after the pretreatment of rats with L-NAME (70 mg/kg), an inhibitor of the NO synthesis activity are presented in Table 5. Animals that received EAPA (100 mg/kg), BPA (200 mg/kg) and L-arginine (200 mg/kg) showed an ulcerated area significantly

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Ulcer index</th>
<th>Volume of gastric juice (mL)</th>
<th>pH</th>
<th>Free acidity (mmol/h)</th>
<th>Total acidity (mmol/h)</th>
<th>Total protein (mg/mL)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle control (0.5 mL, 1% Tween-80, i.d.)</td>
<td>6</td>
<td>0.24±0.01</td>
<td>3.99±0.43</td>
<td>2.79±0.22</td>
<td>0.62±0.01</td>
<td>1.53±0.03</td>
<td>51.48±4.21</td>
<td>0</td>
</tr>
<tr>
<td>EAPA (100 mg/kg, i.d.)</td>
<td>6</td>
<td>0.16±0.02 ▲</td>
<td>2.33±0.25 ▲▲</td>
<td>4.43±0.27 ▲</td>
<td>0.53±0.01 ▲▲</td>
<td>0.72±0.04 ▲▲</td>
<td>69.36±5.38</td>
<td>33.3</td>
</tr>
<tr>
<td>BPA (200 mg/kg, i.d.)</td>
<td>6</td>
<td>0.13±0.02 ▲▲</td>
<td>2.43±0.25 ▲▲</td>
<td>4.13±0.49 ▲▲</td>
<td>0.44±0.02 ▲▲</td>
<td>0.63±0.03 ▲▲</td>
<td>66.06±3.86</td>
<td>45.8</td>
</tr>
<tr>
<td>Ranitidine (50 mg/kg, i.d.)</td>
<td>6</td>
<td>0.12±0.01 ▲ ▲</td>
<td>2.15±0.24 ▲ ▲</td>
<td>4.65±0.40 ▲ ▲</td>
<td>0.42±0.02 ▲ ▲</td>
<td>0.64±0.04 ▲ ▲</td>
<td>77.52±7.45 ▲ ▲</td>
<td>50.0</td>
</tr>
</tbody>
</table>

Data except the inhibition are expressed as mean ± standard error of mean. ▲ $P < 0.05$, ▲▲ $P < 0.01$, vs vehicle control group.

EAPA: ethyl acetate extract; BPA: $n$-butanol extract.

**Figure 3** Effects of EAPA and BPA on macroscopic changes in the pylorus ligation in rats

Results showed that EAPA (100 mg/kg) and BPA (200 mg/kg) treatment prevented the gastric ulcer by reducing the acid secretion as compared to the vehicle control group.

A: Vehicle control group; B: EAPA group; C: BPA group; D: Ranitidine group.

EAPA: ethyl acetate extract; BPA: $n$-butanol extract.

**Figure 4** Microscopic studies of the stomach tissues stained by hematoxylin and eosin from rats submitted to pylorus ligation-induced gastric ulcer assay (Light microscopy, × 100)

A: Control group damaged by pylorus ligation; B: EAPA group treated with pylorus ligation after pretreatment with EAPA; C: BPA group treated with pylorus ligation after pretreatment with BPA; D: Ranitidine group treated with pylorus ligation after pretreatment with ranitidine.

EAPA: ethyl acetate extract; BPA: $n$-butanol extract.
The efficacy of these treatments is questionable due to the development of tolerance and side effects, as well. Thus, the vehicle control group pretreated with L-NAME increased ulceration, L-NAME was unable to reverse the gastroprotection of EAPA and BPA. All these treatments were able to reduce the ulceration of the vehicle control group pretreated with L-NAME significantly (P < 0.01), indicating that NO system is probably not involved with the effect of the extracts.

### Table 5 Effects of EAPA, BPA and L-arginine on ethanol-induced ulcer in L-NAME-pretreated rats

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Ulcer index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle control (0.5 mL, 1% Tween-80, p.o.)</td>
<td>6</td>
<td>0.78±0.05</td>
</tr>
<tr>
<td>EAPA (100 mg/kg, p.o.)</td>
<td>6</td>
<td>0.34±0.03</td>
</tr>
<tr>
<td>BPA (200 mg/kg, p.o.)</td>
<td>6</td>
<td>0.40±0.05</td>
</tr>
<tr>
<td>L-arginine (200 mg/kg, i.p.)</td>
<td>6</td>
<td>0.23±0.03</td>
</tr>
<tr>
<td>L-NAME (70 mg/kg, i.p.)</td>
<td>6</td>
<td>1.04±0.12</td>
</tr>
<tr>
<td>L-NAME (70 mg/kg, i.p.) + EAPA (100 mg/kg, p.o.)</td>
<td>6</td>
<td>0.44±0.02</td>
</tr>
<tr>
<td>L-NAME (70 mg/kg, i.p.) + BPA (200 mg/kg, p.o.)</td>
<td>6</td>
<td>0.46±0.03</td>
</tr>
<tr>
<td>L-arginine (200 mg/kg, i.p.) + L-NAME (70 mg/kg, i.p.)</td>
<td>6</td>
<td>0.69±0.07</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± standard error of mean. *P < 0.05, **P < 0.01, vs vehicle control group; ***P < 0.01, vs L-NAME group; ^P < 0.01, vs L-arginine group.

EAPA: ethyl acetate extract; BPA: n-butanol extract; L-NAME: L-nitro arginine methyl ester.

### 4 Discussion

The therapeutic management of peptic ulcer is based on the use of antacids, anticholinergic, proton pump inhibitors and histamine receptor blockers[29]. However, the development of tolerance and side effects, as well, make the efficacy of these treatments questionable[29]. For these reasons, the search for new drugs is very relevant, and medicinal plants play a key role in these studies, since many extracts and isolated compounds have shown promising results in the treatment and prevention of gastric ulcers[30,31].

To evaluate the gastroprotective effect of the extracts of *P. alba*, the model of acute ulcer induced by indomethacin was first performed, since basic information can be provided by using it, as well as the determination of the therapeutic dose. Antulcer assessment was firstly performed with HAPA in indomethacin-induced gastric lesions, and once its activity was confirmed, the study was carried out to evaluate its organic fractions. The results obtained in the indomethacin model showed that HAPA as well as their EAPA and BPA fractions possesses significant gastroprotective activity. Once all the treatments had their activity determined, we focused on using the most effective treatment with the lowest dose tested. In the present study, EAPA and BPA (100 and 200 mg/kg) respectively, increased the healing of ulcers as demonstrated by a decrease in ulcer index, while HAPA and HPA did not show any significant effect on ulcer index. Indomethacin has better ulcerogenic potential than other NSAIDs[32]. Indomethacin inhibits prostaglandin (PG) synthesis by blocking cyclooxygenase-1, which impairs the mucosal barrier thus rendering gastric mucosa more susceptible to injury[33,34]. Further, cyclooxygenase-1 inhibition induces mucosal injury and inhibition of PG, which activates the neutrophils and the local release of reactive oxygen species and thus starts gastric injury[35]. It is plausible to suggest the possible involvement of PG production or mucus in the antiulcer effect presented by the extracts.

As EAPA and BPA showed antiulcerogenic effect on indomethacin-induced gastric ulceration, we were interested to investigate its effect on other aggressive factors that induced ulceration. Ethanol is one of the most widely used agents in experimental models to evaluate the gastroprotective activity in rats[36,37]. The acute effect of ethanol-induced ulcer has been proved to be its rapid penetration into gastric mucosa, which may cause more mucosal permeability and release of vasoactive mediators such as leukotrienes C4, endothelin-1 and histamine. The vasoactive mediators induce blood flow stasis in mucus membrane circulation, which increases the lesions in mucosa[38,39]. Other factors responsible may be associated with the formation of reactive oxygen species, which cause an imbalance between oxidant and antioxidant cellular process, thus resulting in severe damage to the vascular plexus, and rupture of blood vessels, which contributes to the haemorrhage, tissue necrosis and disrupting the protective mucosal barrier[40,41]. The cytoprotective activity of EAPA and BPA may be due to their direct action on mucus secretion. To study the side effects of *P. alba* on liver, serum AST and ALT were determined in ethanol-induced gastric ulcer model. The control group animals showed increase of serum concentration of these enzymes indicating hepatic injury[42]. Extracts administration decreased the levels of AST and ALT showing their tissue damage-preventing action.

The interference of the extracts on the parameters of gastric secretions was also evaluated using the pylorus ligature method. This method is an important procedure that reveals possible changes of parameters relating to
gastric secretion, e.g., gastric secretion volume, pH, and free and total acidity. Agents that decrease gastric acid secretion and increase pH are effective in protecting the ulcers induced by this method. EAPA and BPA produced a reduction in gastric acid secretion and increase in gastric pH, providing their gastric antisecretory effect.

The role of NO in the modulation of gastric mucosal integrity, and regulation of gastric and mucus secretions along with PG is well established\(^{[43,44]}\). NO is synthesized from L-arginine by nitric oxide synthase (NOS) action\(^{[45]}\). In order to investigate the role of endogenous NO in gastroprotection, L-NAME (NOS inhibitor) was used to evaluate the protective effect of EAPA and BPA on ethanol-induced gastric damage. It was found that L-NAME was unable to abolish the gastroprotective effects of EAPA and BPA. These results indicate that gastro-protection shown by both fractions is not related to NO production. The efficacy of \(P.\) \(alba\) was further substantiated by quantitative histological findings, where a marked decrease in areas of gastric mucosal necrosis could be observed.

\(P.\) \(alba\) hydroalcoholic leaf extract fractions EAPA and BPA contain a number of steroids, alkaloids, tannins, glycosides, flavonoids, and many other chemical constituents. Non-specific gastroprotective activity of the extracts may be due to the combined effect of the different phytoconstituents. Active principles such as flavonoids and tannins have been reported to possess antiulcer property\(^{[47]}\). Tannins act as an astringent and possess vasocostrictive and protein-precipitating effects. Tannins are known to ‘tar’ the outer most layer of the gastric mucosa rendering it less permeable and more resistant to chemical and mechanical injury or irritant\(^{[48,49]}\). Flavonoids are polyphenolic compounds with known antioxidant properties in addition to strengthening the mucosal defence system through stimulation of gastric mucus secretion\(^{[50]}\). The gastroprotective activity of \(P.\) \(alba\) may be due to presence of flavonoids\(^{[51]}\) and other antioxidants\(^{[52]}\). Apart from flavonoids and tannins, the plant is also rich in saponins. Saponins generally act through formation of protective mucosal layer on gastric mucosa and by selectively inhibiting PG F\(_2\)α\(^{[53]}\). These findings from the previous studies defend the potent gastroprotective effect of EAPA and BPA.

In conclusion, the present study provide preliminary data that the sequential fractions of the hydroalcoholic extract of leaves of \(P.\) \(alba\) possess gastroprotective principles, which protect against gastric mucosal damage induced by indomethacin, ethanol and pylorus ligation. The results obtained in this work demonstrate that preparations obtained from \(P.\) \(alba\) could be used for the development of a new drug for the treatment of gastric ulcer. The data obtained so far do not indicate, however, which specific mechanism(s) is (are) responsible for antiulcer activity. Further investigations are required to identify its molecular mechanism and isolate the active components responsible for the antiulcer activity.

5 Competing interests

The authors declare that they have no competing interests.

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