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Anti-ulcerogenic Mechanisms of a Lyophilized Aqueous Extract of *Dalbergia monetaria* L. in Rats, Mice and Guinea-pigs

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Abstract

The decoction of *Dalbergia monetaria* L. is popularly used in Brazil for the treatment of gastric ulcer and the lyophilized aqueous extract (LAE) of *D. monetaria* has significant anti-ulcerogenic activity and inhibits gastric ulcer lesions induced by pylorus-ligature, ethanol and hypothermic-restraint stress. This work was conducted to identify the anti-ulcerogenic mechanisms of action of the LAE of *D. monetaria*. We analysed the effect of the LAE on prostaglandin E_2 (PGE_2) synthesis and on the characteristics (pH, volume and total acid content) of gastric juice. The antagonism between the LAE and histamine or carbachol was also analysed.

The LAE increased gastric mucosal PGE_2 synthesis compared with control ($89{\cdot}7 \pm 21{\cdot}5$ and $52{\cdot}6 \pm 11{\cdot}8\,pg\,mg^{-1}$, respectively) as assayed by enzyme immunoassay in the rat stomach. The LAE reduced the total acid content of gastric juice, but did not modify pH or gastric volume significantly, in Shay rats. Dose–response curves to histamine were shifted to the right in guinea-pig isolated right atria (pD_2 values were $5{\cdot}77 \pm 0{\cdot}2$ and $5{\cdot}42 \pm 0{\cdot}3$, respectively, in the absence and presence of the LAE), with a significant reduction in maximum response ($140 \pm 15{\cdot}1$ and $98 \pm 13{\cdot}0$, respectively). The same effect was observed when the agonist was isoprenaline. The LAE had no effect on the dose–response curve to carbachol in rat fundus strips.

Thus, the protective effect of the LAE on induced gastric lesions might be because of synergistic effects, e.g. increased PGE_2 synthesis and antagonism of H_2 histamine and β-adrenergic receptors, reducing gastric acid secretion. Increased PGE_2 synthesis results in increased protection, and antagonism of H_2 histamine and β-adrenergic receptors reduces aggressive factors against the gastric mucosa.

There have recently been extraordinary advances in the understanding of the pathophysiology and treatment of gastrointestinal disorders (Martin et al 1993). It is generally accepted that peptic ulcers result from imbalance between gastric aggressive factors and mucosal defensive factors (Sun 1974). Robert et al (1983) reported that prostaglandins, even at non-secretory doses, prevented gastric necrosis induced by necrotizing agents and used the term ''cytoprotection'' to denote this unique property of prostaglandins.

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The main aggressive components reported for several decades are acid and pepsin. Thus, peptic ulcer disease has been predominantly treated with antacids, H_2 receptor antagonists and proton pump inhibitors (Wallace et al 1990; Wallace & Granger 1996).

Plants provide an alternative strategy in the search for new drugs for the treatment of gastrointestinal disorders (Lewis & Hanson 1991). The alleged medicinal properties of *Dalbergia monetaria* L. bark in the healing of gastric disorders, anaemia and hepatic disorders have been traditionally reported in Brazilian folk medicine.

Nunes et al (1989) described the isolation and identification of two dimeric proanthocyanidins

from the bark of *D. monetaria* L., which inhibits histidine decarboxylase. Souza Brito et al (1997) showed that the lyophilized aqueous extract (LAE) of *D. monetaria* had significant anti-ulcer activity, inhibiting the gastric ulcer lesions induced by pylorus-ligature, ethanol and hypothermic-restraint stress. In contrast, the LAE afforded no significant protection against the gastric mucosal damage induced by indomethacin.

The aim of this study was to assess the possible mechanisms underlying these pharmacological properties by studying the effect of the LAE on gastric acid secretion and on gastric protection mechanisms. We compared the anti-ulcer activity of the LAE with that of cimetidine, an agent known to inhibit histamine-stimulated gastric acid secretion. By use of isolated preparations we analysed the effects of LAE on H_2 histamine receptors and M_1 muscarinic receptors, both involved in gastric acid secretion. We also investigated whether the anti-ulcer effect of LAE could be mediated by prostaglandin, a mucosal defensive factor.

Materials and Methods

Plant material

D. monetaria occurs naturally in the states of Amazon and Pará in the Northern region of Brazil. Bark of *D. monetaria* was collected in Benfica, near Belém, Pará, Brazil. A voucher specimen (number MG 10·003) was deposited at the herbarium of Museu Paraense Emílio Goeldi in Belém, Pará. The lyophilized aqueous extract of *D. monetaria* was obtained from a 10% infusion of dry and powdered bark.

Animals

Experiments were performed on male and female albino Wistar rats, 150–250 g, on male Swiss mice, 25–35 g, from the Centro de Bioterismo da Universidade Estadual de Campinas (UNICAMP) and on male guinea-pigs, 250–350 g, from the Laboratory of Animal Reproduction (Ministério da Agricultura). When necessary, animals were fasted. Animals received a certified Nuvilab CR-a (Nuvital) diet and water, which was freely available, under standard conditions of 12-h dark–light cycle, $60 \pm 10\%$ humidity and a temperature of $21{\cdot}5 \pm 1{\cdot}0$°C. The protocols were approved by the Institutional Animal Care and Use Committee of UNICAMP which follows the recommendations of the Canadian Council on Animal Care (Olfert et al 1993).

Drugs and chemicals

Cimetidine was from SmithKline (Brazil), Tween 80 from Synth (Brazil), carbamylcholine chloride, carbachol, histamine dihydrochloride, propranolol hydrochloride, isoprenaline hydrochloride and indomethacin from Sigma (St Louis, MO). The chemicals used in the nutritive solutions were all of analytical grade. The LAE was prepared immediately before use and dissolved in 12% Tween 80 before administration to the animals.

Isolated guinea-pig right atria

This experiment was conducted as previously described by Krielaart et al (1990). Male guinea-pigs were killed by cervical dislocation. Hearts were immediately removed, dissected and the atria were mounted under 0·5 g resting tension in 20-mL organ baths containing Krebs–Henseleit solution oxygenated with 95% O_2–5% CO_2 at $36{\cdot}5 \pm 0{\cdot}1$°C. The increase in heart rate (beats min^{-1}) induced by histamine in a cumulative dosing schedule was measured with an isometric transducer (Narco Bio-System) coupled to a polygraph (Narco Bio-System). The β-adrenoceptors were blocked with 1 μM propranolol previously added to the Krebs–Henseleit solution. After obtaining the cumulative dose–response curve for histamine the preparations were washed for 15 min with four changes of bathing solution. The atria were pre-equilibrated with one of four different concentrations (40, 120, 400 or 1200 $\mu g\,mL^{-1}$) of the LAE for 60 min before determination of another dose–response curve for the same agonist. The same procedure was used when the agonist was isoprenaline. In the last experiment the cumulative dose–response curves for isoprenaline were obtained in the presence of 3 μM cimetidine, also added to the Krebs–Henseleit solution. In this experiment the atrium was pre-equilibrated with one of two different concentrations (40 or 1200 $\mu g\,mL^{-1}$) of the LAE.

Isolated rat fundus stomach

Male and female albino Wistar rats were deprived of food 8 h before the experiment, as previously described by Korolkiewicz et al (1997). Animals were killed by cervical dislocation. The abdomen was opened by midline incision, the stomach excised, and the fundus dissected out and cut into longitudinal strips according to the method described by Vane (1957). The strips were placed in Krebs–Henseleit solution as described for the atrium experiment. One end of the strip was attached to a fixed support and the free end to a lever connected to an isometric transducer coupled

to a polygraph. The contractions induced by carbachol were measured as the tension developed by the tissue. The H_2 histaminergic receptor was blocked with 1 μM cimetidine present in the Krebs–Henseleit solution. Tissues were left to equilibrate for 60 min before the beginning of the experiment. The nutritive solution was changed every 10 min, except during the 30 min of contact with the LAE. Dose–response curves for carbachol were obtained in the absence and presence of different concentrations (40 and 1200 $\mu g\,mL^{-1}$) of the LAE.

Determination of prostaglandin synthesis

All rats were deprived of food for 24 h before the experiment and all experiments were performed between 0900 and 1100 h. The groups received 12% Tween 80 (control, administered orally), indomethacin ($20\,mg\,kg^{-1}$, administered subcutaneously), the LAE ($1\,g\,kg^{-1}$, administered orally), or the LAE plus indomethacin. Indomethacin was dissolved in 5% sodium bicarbonate solution. Animals were killed by cervical dislocation 30 min after treatment and the abdomen was opened. A sample of the corpus (full thickness) was excised, weighed, and suspended in sodium phosphate buffer (10 mM, pH 7·4; 1 mL). The tissue was finely minced with scissors and incubated at 37°C for 20 min. Prostaglandin E_2 (PGE_2) in the buffer was measured by enzyme immunoassay (EIA; RPN222 kit, Amersham). The optical density was read at 450 nm as previously described by Curtis et al (1995).

Determination of gastric secretion

Seventeen mice were randomly divided into three groups and fasted for 24 h with free access to water. The assay was performed by the method of Shay et al (1945), with modifications. Pylorus ligature was performed immediately after intraduodenal administration of the LAE ($1\,g\,kg^{-1}$), of cimetidine ($100\,mg\,kg^{-1}$) as positive control, or of vehicle ($10\,mL\,kg^{-1}$) as negative control. The animals were killed 3 h later, the abdomen was opened, and another ligature was placed around the oesophagus close to the diaphragm. The stomach was removed and its contents were measured and drained into a graduated tube which was centrifuged at $2000\,rev\,min^{-1}$ for 10 min. The pH was recorded with a digital pH meter (PA 200; Marconi, Brazil) and the total acid content of gastric contents was also determined in the supernatant volume by titration to pH 7·0 with 0·01 M NaOH, by use of a digital burette (E.M., Hirschmann Technicolor, Germany).

Statistical analysis

Results are expressed as means ± s.e.m. or s.d. Statistical significance was determined by one-way analysis of variance followed by Dunnett, Fisher or Scheffé test, with the level of significance set at $P < 0{\cdot}05$. All statistical analyses were performed by use of Statistic 5.0 software (Systat, USA).

Results and Discussion

We have previously demonstrated the anti-ulcerogenic properties of the lyophilized aqueous extract (LAE) of *D. monetaria*. The LAE inhibits the gastric lesions induced in rats by ethanol, hypothermic-restraint stress and pylorus-ligature, but not the ulcers induced by indomethacin (Souza Brito et al 1997). We believe these results were because of a local effect of the extract. The current results (Table 1) show that both LAE and cimetidine administered intraduodenally induced a marked reduction in total gastric juice acidity. An increase in total gastric juice volume was observed only with cimetidine. These results confirm that both drugs have a systemic action rather than a local effect.

Histamine is thought to play a central role in the regulation of gastric acid secretion (Andersson et al 1996). Studies have suggested that the release of histamine is a major regulatory event in the stimulation of acid secretion and increased histamine secretion might be associated with mucosal damage

Table 1. The effects of the lyophilized aqueous extract of *D. monetaria* on the biochemical parameters of the gastric juice obtained from pylorus-ligated mice.

Treatment	Dose ($mg\,kg^{-1}$)	n	Juice volume (mL)	pH	Total acid content ($mEq\,mL^{-1}$)
Control	–	4	0·36 ± 0·03	3·67 ± 0·52	6·02 ± 1·58
Cimetidine	100	5	0·65 ± 0·23	6·13 ± 0·73*	1·87 ± 0·62*
Extract	1000	8	0·37 ± 0·13	4·38 ± 1·06	2·51 ± 1·49*

Values are means ± s.d. of results from 4–8 animals (n). Analysis of variance $F_{2,20}$ volume = 7·399, $P < 0{\cdot}05$; pH = 14·05, $P < 0{\cdot}05$; $[H^+] = 17{\cdot}36$, $P < 0{\cdot}05$. Scheffé test $^*P < 0{\cdot}05$.

Table 2. Maximum response, pD_2† and basal rate obtained from dose–response curves to histamine and to isoprenaline in the absence and presence of the lyophilized aqueous extract (LAE) of *D. monetaria* applied to guinea-pig isolated right atria.

Parameter	Histamine					Isoprenaline		
	Lyophilized aqueous extract ($\mu g\,mL^{-1}$)					Lyophilized aqueous extract ($\mu g\,mL^{-1}$)		
	0	40	120	400	1200	0	40	1200
pD_2	5·77 ± 0·22	5·67 ± 0·10	5·49 ± 0·16	5·42 ± 0·30*	5·52 ± 0·09	8·17 ± 0·35	8·32 ± 0·15	7·72 ± 0·22*
Basal rate (beats min^{-1})	186 ± 24	164 ± 23	184 ± 8·9	178 ± 13	130 ± 19*	204·6 ± 17	200 ± 27	160 ± 24*
Maximum response (beats min^{-1})	140 ± 15	144 ± 23	118 ± 8·4	104 ± 15*	98 ± 13*	173 ± 26	179 ± 13	113 ± 16*
n	8	5	5	5	5	11	7	6

Mean ± s.d. −log EC50 where EC50 is the dose inducing 50% contraction. Histamine analysis of variance: $F_{4,33}$ maximum response = 9·83, $P < 0·05$; pD_2 = 3·47, $P < 0·05$; basal rate = 7·83, $P < 0·05$. Dunnett test $*P < 0·05$. Isoprenaline analysis of variance: $F_{2,27}$ maximum response = 19·62, $P < 0·05$; pD_2 = 8·38, $P < 0·05$; basal rate = 5·13, $P < 0·05$. Dunnett Test $*P < 0·05$.

(Sachs et al 1994). Acetylcholine also stimulates secretion of gastric acid either directly by M_1 receptors in parietal cells or indirectly by stimulation of histamine and gastrin secretion by gastric mucosa. We performed a series of functional studies in which we analysed the response of isolated preparations to different agonists.

In spontaneously beating guinea-pig right atria (Figure 1A) dose–response curves to histamine were shifted to the right in a non-dose-dependent manner in the presence of the LAE. In addition, the maximum response to histamine was reduced by the LAE (400 and 1200 $\mu g\,mL^{-1}$) and the highest dose induced an additional decrease of basal atrial rate (Table 2). Krielaart et al (1990) have already reported a similar effect of histamine-receptor antagonists on guinea-pig right atria characterized by a decrease in the maximum response to the agonist. However, a similar effect was observed when the agonist was isoprenaline, a β-adrenergic receptor agonist, which is non-selective for subtype (Figure 1B). These results indicate that the action of the LAE on the guinea-pig right atrium response to chronotropic agents is non-specific.

The same rationale was used to analyse the effects of the LAE on M_1 receptors in isolated rat stomach fundus (Figure 2). Dose–response curves to carbachol were also shifted to the right together with a decrease in maximum response but the values obtained (pD_2 (= −log EC50 where EC50 is

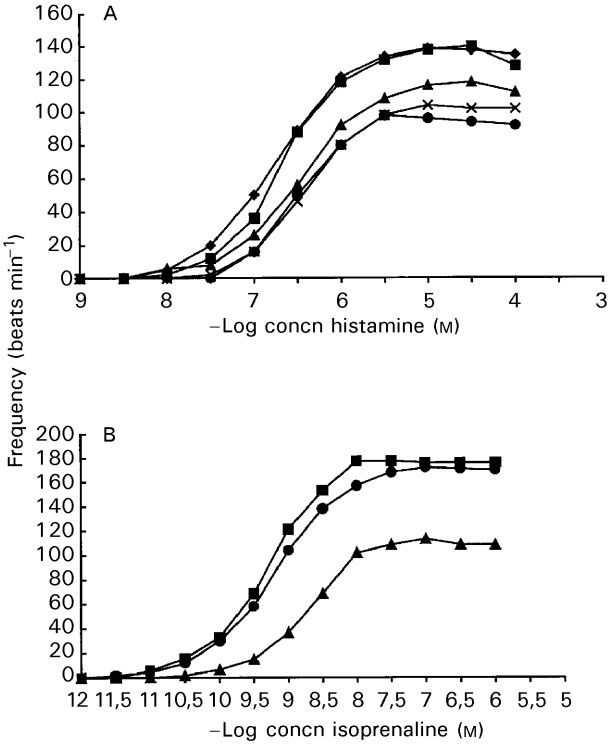

Figure 1. Cumulative dose–response curves to histamine (A) and to isoprenaline (B) in the absence and presence of different concentrations of the lyophilized aqueous extract (LAE) of *D. monetaria* (0, ■ 40, ▲ 120, ◆ 400, ● 1200 $\mu g\,mL^{-1}$ for A and ● 0, ■ 40, ▲ 1200 $\mu g\,mL^{-1}$ for B). Each point represents the mean of results from 5–8 experiments for histamine and 6–11 experiments for isoprenaline.

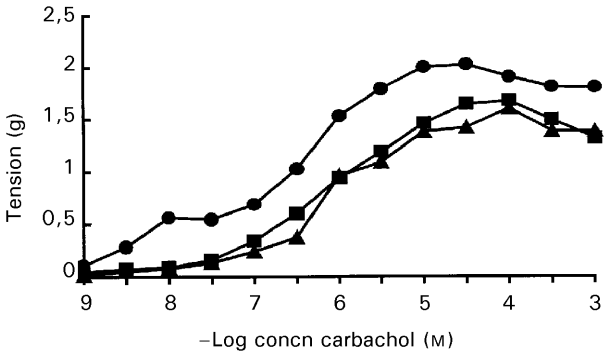

Figure 2. Cumulative dose–response curves to carbachol in the absence and presence of different concentrations of the lyophilized aqueous extract (LAE) of *D. monetaria* (● 0, ■ 120, ▲ 1200 $\mu g\,mL^{-1}$). Each point is the mean of results from 5–9 experiments.

Table 3. Maximum response and pD_2† for carbachol applied to rat isolated gastric fundus strips in the absence and presence of the lyophilized aqueous extract of *D. monetaria*.

	Lyophilized aqueous extract ($\mu g\,mL^{-1}$)		
Antagonist-LAE ($\mu g\,mL^{-1}$)	0	120	1200
pD_2	6·39 ± 0·47	6·21 ± 0·13	6·08 ± 0·45
Maximum response (g)	2·15 ± 0·54	1·72 ± 0·13	1·61 ± 0·001
n	9	5	5

Mean ± s.d. −log EC50. Analysis of variance: $F_{2,22}$ maximum response = 2·12, $P > 0·05$; $pD_2 = 1·089$, $P > 0·05$.

the dose inducing 50% contraction) and maximum response) were not statistically significant (Table 3).

Although the involvement of histamine at the H_2 receptor level or acetylcholine at the M_1 receptor level in stimulating gastric acid secretion is well documented (Hirschowitz et al 1995), it has recently been shown that β-adrenoceptor agonists stimulate acid output in isolated rat stomach and that these actions can be antagonized by β-blockers. These observations suggest that blocking the stomach β-adrenoceptors not only strengthens the mucosal barrier but could also alleviate the aggressive action of acid and perhaps pepsin on the gastric mucosa (Canfield et al 1981; Kaan & Cho 1997).

These functional studies revealed the same pattern of effect of the LAE on the response of different tissues to agonists, suggesting independence of tissue and receptor. However, there is no doubt that the LAE interferes with gastric acid secretion because all the responses analysed were related to it.

Among the many factors that might contribute to the protective actions of prostaglandins in the stomach are stimulation of the secretion of mucus and bicarbonate, maintenance of gastric blood flow during exposure to an irritant, and inhibition of inflammatory-mediator release from mast cells and of free radical production (Motilva et al 1996). We previously reported that the LAE did not increase mucus secretion (Souza Brito et al 1997).

Whilst looking for other possible mechanisms that increase mucosal protective factors we investigated the effect of the LAE on PGE_2 production. The gastric protective effect of prostaglandins has been related to the ulceration induced by cyclooxygenase inhibitors or by non-steroidal anti-inflammatory drugs (Curtis et al 1995; Eberhart & Dubois 1995; Threvethick et al 1995).

Our data, listed in Table 4, show that the LAE increases PGE_2 production, an effect which is abolished by pretreatment with indomethacin. Therefore, the LAE has an anti-ulcerogenic effect by reducing the total acid content of gastric juice and enhancing PGE_2 synthesis. These two effects are observed when the LAE is administered before lesion induction, which means that the LAE has a preventive anti-ulcerogenic effect.

No synthetic drugs with cytoprotective properties and the ability to reduce gastric acid secretion are currently available. In contrast, plant polysaccharides with both properties have been described (Sun et al 1991).

In conclusion, these results, taken together, suggest that the LAE has a beneficial preventive effect on gastric ulcers, mainly by suppressing acid secretion by non-competitive and non-specific antagonism of the receptors involved in gastric acid secretion and by protecting the gastric mucosa by increasing production of PGE_2.

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Table 4. Effects of oral administration of the lyophilized aqueous extract (LAE) of *D. monetaria* and of indomethacin on gastric prostaglandin E_2 (PGE_2) production in rats.

Treatment	Dose ($mL\,kg^{-1}$)	n	PGE_2 synthesis ($pg\,mL^{-1}$)	Increase (%)
Tween	–	5	52·6 ± 11·8	–
Tween + indomethacin	–	4	32·5 ± 13·1	−38
Indomethacin	30	4	28·8 ± 13·5*	−45
LAE	100	6	89·7 ± 21·5*	71
LAE + indomethacin	100	2	16·1 ± 0·57*	−69

Values are means ± s.d. of results from 2–6 animals). Analysis of variance $F_{(4,26)} = 13·8$, $P < 0·0001$. Fisher's test (control group relationship) $*P < 0·05$.

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