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Evaluation of Gastric Anti-ulcer Activity in a Hydro-ethanolic Extract from *Kielmeyera coriacea*

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ABSTRACT

The antiulcer activity of a hydro-ethanolic extract prepared from the stems of Kielmeyera coriacea Mart. (Guttiferae) was evaluated in rats employing the ethanol-acid, acute stress and Indomethacin models to induce experimental gastric ulcers. Treatment with K coriacea hydro-ethanolic extract provided significant antiulcer protection in the ethanol-acid and Indomethacin models, but not in the acute stress model. These results suggested that the K coriacea hydro-ethanolic extract increased resistance to necrotizing agents, providing a direct, protective effect on the gastric mucosa.

Key words: Acute stress, anti-ulcer activity, ethanol-acid, Indomethacin, Kielmeyera coriacea Mart. (Guttiferae)

INTRODUCTION

Kielmeyera coriacea Mart. (Guttiferae) is a plant species typically found among the Brazilian cerrado vegetation, and is popularly known as the "pau santo". A decoction from the stems is traditionally used to treat various tropical diseases, including schistosomasis, leishmaniasis, malaria and fungal and bacterial infections (Alves *et al*, 2000; Ferri, 1969). Xanthones, triterpenes and biphenyl derivatives have been isolated from this plant, and exhibited antifungal activity against *Cladosporium cucumerinum* and *Candida albicans* (Cortez et al., 1998). A hydro-ethanolic extract of *K coriacea* leaves, when administered chronically by gavage, exhibited a notable anxiolytic effect in rats submitted to the elevated plus maze test (Audi et al., 2002). Despite these reports, knowledge of the pharmacological properties of *K coriacea* is limited, and screening studies are necessary to reveal the medicinal properties of the plant.

Recently, widespread effort have been launched to identify novel anti-ulcer drugs from natural resources. A number of models are available in which to test substances for their anti-ulcer effects. Here, we report on the effect of a hydro-ethanolic extract from *K coriacea* stems on gastric lesion induced in different animal models employing necrotizing or stressor agents.

MATERIALS AND METHODS

Plant material

K coriacea was collected near Mogi-Guaçu (São Paulo, Brazil) in July, 1999. A voucher specimen (#SP298463) was deposited with the Herbarium of

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the State Botanical Institute, São Paulo, Brazil, and the species identification was performed by Dr Maria Claudia Young from the same institution.

Extract Preparation

The stems of *K coriacea* Mart were crushed and powdered using a grinding mill, a standardized extract being prepared by maceration. The extract was concentrated by evaporation to 10% of its volume and was then lyophilized. Each 100 g of powdered stem yielded 17 g of lyophilized extract. The active solution was prepared by dissolving the lyophilized extract in saline (0.9% NaCl). The doses employed ranged from 30 to 120 mg/kg, applied in rats.

Animals

Male, Wistar rats (200-250 g) provided by the Animal Housing, Facility of Maringá State University were housed in polyethylene-walled cages in groups of five, with food and water *ad libitum*. The animals were kept on a 12 h light: 12 h dark regime (lights on from 7:00 h to 19:00 h) at 23 °C prior to the experiments.

Treatments

Doses of 30, 60 or 120 mg/kg *of K coriacea* extract were prepared as aqueous suspensions. Cimetidine (32 mg/kg) and 0.9% NaCl were used as the reference drug and control vehicle, respectively. All treatments were administered orally (gavage), 30 min before the procedures.

Reagents

The following drugs were used: Cimetidine (Galena), Indomethacin (Sigma)**,** HCl (Synth).

Procedure to induce acute, gastric, mucosal lesions

The rats were deprived of food for 24 h, although water was allowed. Immediately after each procedure, the animals were killed and their stomachs removed, opened, and the inner lining examined. The gastric lesions were counted, and an ulcerative index (UI) was calculated for each animal as follows:

$$UI = (n \text{ lesion I}) + (n \text{ lesion II}) 2 + (n \text{ lesion III}) 3$$

Where:

I = presence of edema, hyperemia and single, submucosal, punctiform hemorrages (petechiae);

II = presence of submucosal, hemorrhagic lesions with small erosions;

III = presence of deep ulcer with erosions and invasive lesions (Szelenyl and Thiemer, 1978).

Ethanol-acid induced ulcer

The extract of *K coriacea* in different doses, Cimetidine or 0.9% NaCl were administered to rats 30 min before ethanol-acid (25 mg/kg) treatment (0.3 M HCl in 60% ethanol). All treatments were made by gavage. After 1 h, the animals were killed and the gastric lesions were counted (Mizui and Douteuchi, 1983).

Acute stress-induced ulcer

Acute, gastric lesions were induced by stress according to the model of Nagura (1972), modified by Bacchi (1988). After oral administration of 0.9% NaCl, Cimetidine and different doses of *K coriacea* extract, each rat was immobilized in a cylindrical cage and vertically immersed in water to the level of the xiphoid process for 17 h at 23°-25°C. After this, the animals were immediately killed, their stomachs removed, and the gastric lesions were counted.

Indomethacin-induced ulcer

Indomethacin (20 mg/kg) was administered subcutaneously to unanesthetized rats, following the method of Aguwa and Mittal (1981). The animals were killed 7 h later to calculate the ulcerative index. *K coriacea* extract, Cimetidine or 0.9% NaCl were administered 30 min before Indomethacin treatment.

Acute toxicity

Acute toxicity studies of *K coriacea* extract were performed on mice, and the lethal dose was estimated using the method described by Miller and Tainter (1944). Increasing doses of extract were individually administered to groups of 10 animals which were observed daily for 7 days.

Statistical Analysis

The results are expressed as the mean ± SEM for each group. Statistical differences were evaluated using a one-way analysis of variance (ANOVA) followed by Dunnett´s post-hoc test. Differences were considered to be statistically significant at $P \leq 0.05$.

RESULTS

Gastric mucosal damage, induced using the stress model was not affected by different doses of *K coriacea* extract ($F(4,25)=4.79$, $p=0.0052$) (Fig 1). Cimetidine (32 mg/kg), the reference drug, significantly reduced the ulcerative index in all three models of gastric mucosal lesion.

Gastric mucosal lesions, induced using ethanol-acid were significantly reduced by the 30- and 120-mg/kg doses of extract (Fig 2), ($F(4,31)=9.182$, $p<0.0001$) compared to the control group. Gastric lesions induced by Indomethacin (Fig 3) were significantly reduced only by the 30-mg/kg dose of extract ($F(4,25)= 8.746$, $p<0.0001$) compared to the control group. Administration of *K coriacea* extract in doses up to 1200 mg/kg produced no signs of toxicity in mice (data not shown).

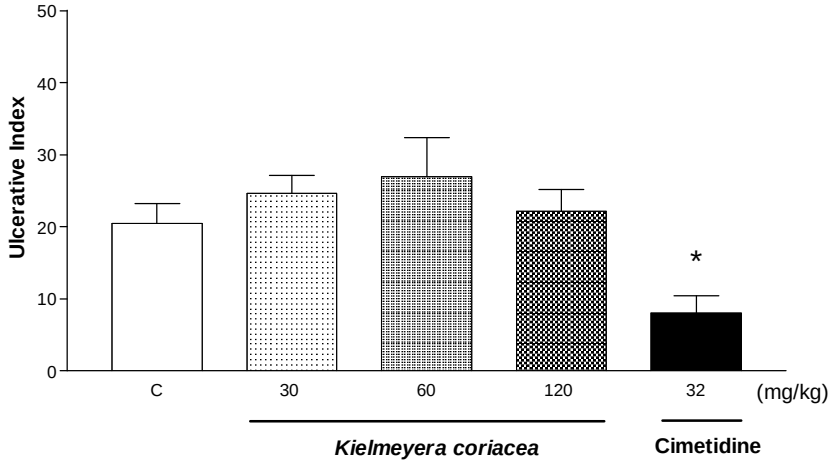

Figure 1 - Mean ± SEM of ulcerative index obtained with oral doses of 30, 60 and 120 mg/kg of hydroethanolic extract of *Kielmeyera coriacea*, control (C, 0.9% NaCl) and cimetidine (32 mg/kg) induced by stress model. ANOVA and Dunnet's test were utilized for comparisions, (*$p< 0.05$), (n=6).

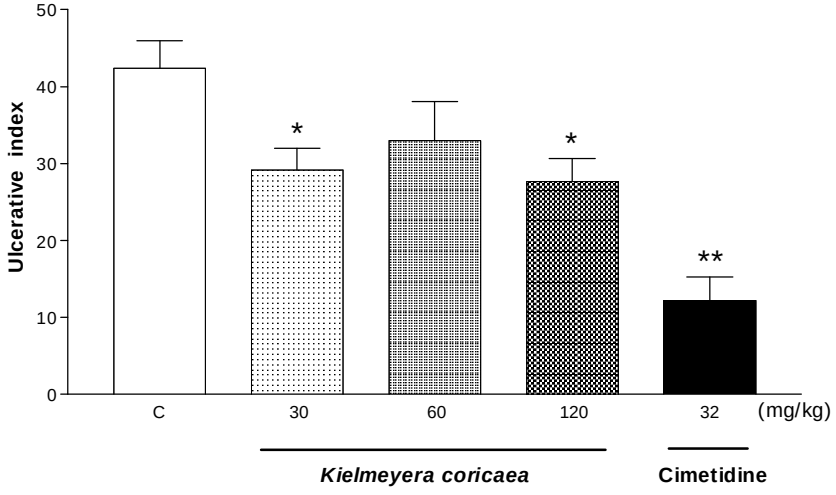

Figure 2 - Mean ± SEM of ulcerative index obtained with hydroethanolic extract of *Kielmeyera coriacea* (30, 60 and 120 mg/kg, p.o.), control (C, 0.9% NaCl) and cimetidine (32 mg/kg) on ethanol-acid model. ANOVA and Dunnett´s test were utilized for comparisions, (*$p<0.05$, **$p<0.01$), (n=6-8).

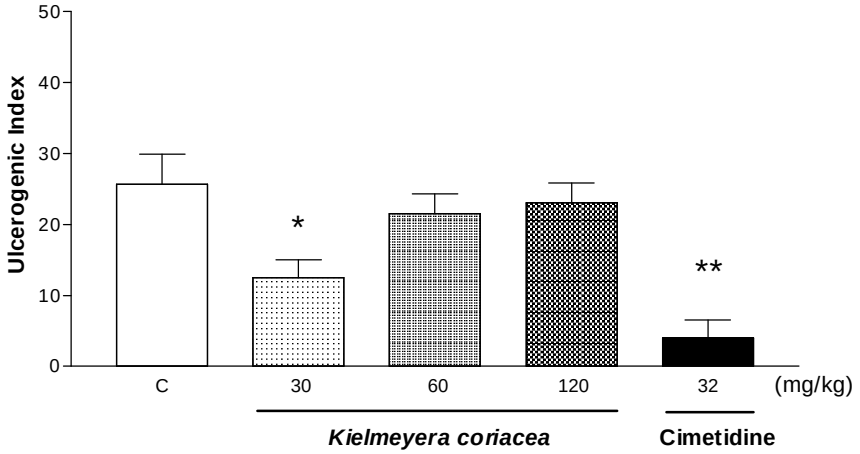

Figure 3 - Mean ± SEM of ulcerative index obtained with diferent doses of extract of *Kielmeyera coriacea*, control (C, 0.9% NaCl) and cimetidine (32 mg/kg) on indomethacin model. ANOVA and Dunnett´s test were utilized for comparisions, (*p<0.05, **p<0.01), (n=6).

DISCUSSION

This study revealed a significant anti-ulcer effect of a hydro-ethanolic extract from *K coriacea* in experimental models of gastric lesion induced by ethanol-acid and by a non-steroidal, anti-inflammatory drug, Indometacin. Under our experimental conditions, different extract doses did not alter the gastric mucosal lesions in the stress model compared to the control.

Anti-inflammatory drugs like Indomethacin administered in toxic doses (20 mg/kg), produce visible gastric ulcers in animals. Indomethacin is a potent inhibitor of prostaglandin biosynthesis (Vane, 1971). Prostaglandins are known to play an important role in maintaining mucosal integrity. An Increase in certain endogenous prostaglandins can enhance gastric mucosal resistance to ulcerogenic agents (Robert, 1979). The mechanisms involved in prostaglandin action are multiple, including stimulation of mucus and bicarbonate output (Hogan et al., 1994), gastric mucosal blood flow (Gaskil et al, 1982), decreasing gastric motility, increasing the release of endogenous mediators of gastric injury-vasoactive amines and leucotrienes and stimulation of cellular growth and repair (Hawkey and Rantim, 1985). In the present study, the effect of the extract on prostaglandin biosynthesis was not evaluated, but an increase in resistance to the necrotizing effect of Indomethacin was noted. Ethanol-acid causes more severe gastric mucosal ulceration. The ulcers are caused either by a direct effect of the ethanol-acid solution on the gastric epithelium, or are modulated indirectly by the release of vasoactive products from mast cells (Szabo, 1987), resulting in the release of mediators such as histamine (Oates and Hakkinen, 1988).

Endogenous histamine formation and its release from mast cells in the gastric mucosa also have been implicated in the pathogenesis of gastric ulcers produced by acute stress (Guth and Hall, 1960). Levine and Senay (1968) showed that stress increases histidine decarboxylase activity in the gastric mucosa, and that the degree of increase correlated positively with the number and severity of lesions. Yet, it has been shown vascular changes in ethanol-induced gastric mucosal injury and severe damage in such injury is associated with extensive lesions of mucosal capillaries, increased vascular permeability and reduction of blood flow in mucosa (Gaskil et al, 1982). In our experiments, the extract prevented acute, gastric mucosal injury induced by ethanol-acid and indomethacin. In the ethanol-acid method, the protective action was produced at a lowest and at a highest dose, but not a intermediate dose of extract. The specific mechanisms underlying this action in unknown. However, as a first step, the extract should be fractioned and further studied. The extract did show a significant, cytoprotective effect against the gastric lesions induced by necrotizing agents, which suggests a direct, protective effect on the gastric mucosa. In contrast,

the extract did not decrease the ulcerative index in the stress model at any dose used.

The mechanisms underlying the protective action of the extract against ethanol and indomethacin induced gastric lesions are unclear. Further studies using more specific methods are required to explore the compounds responsible for the protective effect, and the mechanism of this activity. Chronic toxicity studies are also in progress.

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RESUMO

A atividade antiulcerogênica do extrato hidroetanólico de caule de *Kielmeyera coriacea* Mart. (Guttiferae) foi avaliada em ratos por meio de três modelos experimentais: etanol-ácido, indometacina e estresse agudo. O índice ulcerativo observado após o tratamento com o extrato de *Kielmeyera coriacea* foi comparado com a droga de referência, cimetidina. O tratamento com o extrato mostrou significante atividade anti-ulcerogênica nos modelos de indução de lesões de mucosa gástrica produzidas por etanol-ácido e indometacina, mas não contra úlcera induzida pelo modelo de estresse agudo. Etanol-ácido e agentes antiinflamatórios, como a indometacina, são compostos que produzem úlcera de mucosa gástrica. Os resultados deste estudo sugerem uma atividade protetora de mucosa gástrica para o extrato de *Kielmeyera coriacea*

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