

Preliminary Studies of Anti-Ulcerogenic Effect of *Aster squamatus* Leaves Hydroalcoholic Extract on Various Ulcer Models in Rats

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SUMMARY. The anti-ulcerogenic effect of crude hydroalcoholic extract (70%) of *Aster squamatus* leaves (CHE) was tested against ethanol-, indomethacin-, and cold stress- induced ulcers. The CHE (500 and 1000 mg/kg) reduced the lesion index (LI) and the number of ulcers (NU) in ethanol-induced ulcers. The dose of 1000 mg/kg reduced the NU in the model of the indomethacin, and also reduced the LI and NU in the stress model. According to the obtained data, several action mechanisms may be involved in the gastric protection. Subsequent studies should be made trying to evidence the substances involved in *Aster squamatus* anti-ulcerogenic effect.

RESUMEN. "Estudio preliminar del efecto anti-ulcerogénico del extracto hidroalcohólico de las hojas de *Aster squamatus* en varios modelos de úlcera en ratas". Se comprobó el efecto anti-ulcerogénico del extracto crudo hidroalcohólico (70%) de las hojas de *Aster squamatus* (ECH) frente a úlceras inducidas por etanol, indometacina y estrés provocado por el frío. El ECH (500 y 1000mg/kg) redujo el índice de la lesión (IL) y el número de úlceras (NU) inducido por etanol. La dosis de 1000 mg/kg redujo el NU en el modelo de la indometacina y también redujo el IL e NU en el modelo provocado por estrés a frío. De acuerdo con los datos obtenidos, varios mecanismos de acción pueden estar involucrados en la protección gástrica. Subsiguientes estudios deben ser realizados para intentar poner en evidencia las sustancias involucradas en el efecto anti-ulcerogénico del *Aster squamatus*.

INTRODUCTION

Aster squamatus (Spreng.) Hieron. (Asteraceae) is a species native of Rio Grande do Sul state, Southern Brazil. This plant is commonly used due to its antidiarrhoeic, antineoplastic and cicatrizing effects ¹. Phytochemical studies of *A. squamatus* have revealed the presence of steroids and triterpenes, flavonoids, terpenes, phenols, amino-groups, saponins, and tannins ². Leaf infusions seem to increase intestinal water and ion absorption as well as decrease of the gastrointestinal propulsion ¹.

The present study was designed to evaluate the antiulcerogenic activity of the crude hydroalcoholic extract (CHE) in different experimental models in rats.

MATERIALS AND METHODS

Plant material and extract preparation

The plant (*A. squamatus*) was collected in Santa Maria, Rio Grande do Sul state, on March 2001. A voucher specimen was registered in the herbarium of the Department of Biology of the Universidade Federal de Santa Maria (SMDB nº 7609). The leaves were maintained in a ventilated oven (40 °C) for drying and stabilization. The material was further pulverized in a Willye mill. To prepare the CHE, 50 g of leaves were immersed in 500 mL of ethanol and water (70-30%), for 48-72 h, with occasional stirring. The obtained extract was filtered and concentrated in a Fisaton 802D water-bath (40 °C). Then, the concentrated extract was maintained in an oven

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at the same temperature until the weight remained constant to determine yield.

Ethanol induced ulcer

The procedures for ethanol-induced ulcers were an adaptation of the method of Robert *et al.* ³. After a 24 h fast, male Wistar rats (200-300 g, groups of 6 animals) received by gavage (0.5 mL/100g) 500 and 1000 mg/kg CHE. Control animals were similarly treated with vehicle (distilled water) only. Sixty minutes after this procedure, every animal received by gavage 1 mL of ethanol 60%. One hour later the rats were killed, the stomachs removed, and opened along the small curvature to assess the lesion index (LI) (lesions preceding ulcers) and the number of ulcers (NU). The LI of each animal was calculated by adding the following values obtained in the observation of mucosa considering the lesion degree produced: light (1 point), moderate (2 points) or intense (3 points), evaluating the discoloration of mucosa, the loss of pleats, the petechial presence, the edema presence, the hemorrhages and the mucus loss. NU was determined by direct count of the smaller lesions or same to 1 mm. When larger, the lesions were quantified considering 1,5 points for mm.

Indomethacin induced ulcer

Groups of six male rats (24 h fasting) received by gavage (0.5 mL/100g) distilled water (control), 500 and 1000 mg/kg CHE, and standard (ranitidine 50 mg/kg). After 60 minutes indomethacin was injected subcutaneously (30

mg/kg), according to Djahanguiri ⁴. The rats were killed six hours later and the LI and NU determined.

Stress induced ulcer

Cold stress induced ulcers were produced according to Senay & Levine ⁵. After a 36 h fast, groups of six female Wistar rats (200-300g) received by gavage (0.5 mL/100g) distilled water (control), 500 and 1000 mg/kg CHE, and standard (ranitidine 50 mg/kg). After 60 minutes the animals were immobilized individually at 4 °C for 4 h. Then the rats were killed, the stomachs removed, and the LI and NU determined.

Statistical analysis

All values are expressed as mean ± SEM, statistical differences among the experimental groups were assessed by one-way analysis of variance and the Dunnet test, with the aid of the Instat 2.06 test. The minimum significant level was P < 0.05.

RESULTS

In ethanol induced ulcer model (Table 1), the CHE in doses of 500 and 1000 mg/kg reduced the LI in 52.5% (3.16 ± 0.47) and 47.4 % (3.5 ± 0.22), respectively, when compared with the control group (6.66 ± 0.66). These doses of the CHE also decreased in 99.4% (0.33 ± 0.33) and 100%, respectively, the NU with relation to the control group (61.6 ± 11.9).

In indomethacin induced ulcer model (Table 2), the CHE did not showed any significant ef-

Treatment	N	Lesion Index	Number of ulcers
Control (vehicle)	6	6.66 ± 0.66	61.6 ± 11.9
CHE 500 mg/Kg	6	3.16 ± 0.47*	0.33 ± 0.33*
CHE 1000 mg/Kg	6	3.5 ± 0.22*	*

Table 1. Effect of CHE from *A. squamatus* on ethanol-induced gastric ulcers.

* Significantly different from control (P<0.05).

Treatment	N	Lesion Index	Number of ulcers
Control (vehicle)	6	4.5 ± 0.42	13.5 ± 4.3
Ranitidine 50 mg/kg	6	2.33 ± 0.33*	*
CHE 500 mg/Kg	6	4.5 ± 0.56	3.83 ± 3.27
CHE 1000 mg/Kg	6	4.5 ± 0.71	0.5 ± 0.34*

Table 2. Effect of CHE from *A. squamatus* on indomethacin-induced gastric ulcers.

* Significantly different from control (P<0.05).

Treatment	N	Lesion Index	Number of ulcers
Control (vehicle)	6	6.6 ± 0.8	5.66 ± 1.6
Ranitidine 50 mg/kg	6	3.83 ± 0.3*	1.33 ± 0.95*
CHE 500 mg/Kg	6	6.16 ± 0.3	7 ± 2.84
CHE 1000 mg/Kg	6	3.8 ± 0.47*	0.5 ± 0.5*

Table 3. Effect of CHE from *A. squamatus* on stress-induced gastric ulcers.

* Significantly different from control (P<0.05).

fect on the LI. The highest dose of the reduced the NU in 96.3% (0.5 ± 0.34) when compared with the control group (13.5 ± 4.3) CHE.

In cold restraint-induced stress ulcer (Table 3), the CHE in dose of 1000 mg/kg reduced the LI in 42.5% (3.8 ± 0.47) when compared with the control group (6.6 ± 0.8), and the NU in 91% (0.5 ± 0.5) in relation to the control group (5.66 ± 1.6).

DISCUSSION

Ethanol is a corrosive agent to the rat gastric mucosa. It promotes superficial cellular necrosis and release of histamine and leucotriene C_4 . These tissue-derived mediators act on gastric microvasculature, starting events that result in mucosal and possibly submucosal tissue destruction⁶. The results of our study suggested that *A. squamatus* CHE prevented the necrotic action of these mediators on the gastric microvasculature. In this study, ranitidine was not used as standard substance, since the same is not effective in the model of lesions induced by ethanol⁶.

Indomethacin inhibits gastroduodenal bicarbonate secretion due to the reduction of endogenous prostaglandin biosynthesis as well as gastric mucosal blood flow. When an anti-ulcer agent reduces the effect of indomethacin, probably its effect is through the mediation of endogenous prostaglandins⁷. As *A. squamatus* CHE (1000 mg/kg) showed effect on indomethacin-

induced lesions, it is suggested that its action can also be related to the cytoprotection mediated by prostaglandins. However, CHE showed significant effect only on the NU, which suggested that this dose didn't present enough effect for a good gastric protection; so, subsequent studies using higher doses should be carried on.

An increase of the acid secretion is usually associated with the occurrence of stress-induced lesions⁸. Typical antisecretory drugs, which are H₂-receptor antagonists, inhibited stress-induced ulcers^{9,10}. As *A. squamatus* CHE in a dose of 1000 mg/kg reduced the LI and NU of cold stress induced ulcers, additional studies are needed to determine if the CHE presents effect on the gastric secretion.

The different action mechanisms presented by *A. squamatus* in the ulcer models can be related to the several chemical components of this plant, as flavonoids and saponins, mentioned by several authors as possible anti-ulcerogenic agents¹⁰⁻¹². The dose of 1000 mg/kg showed larger effectiveness, since it protected in the three models of gastric lesions.

CONCLUSIONS

The results of the present study suggest that the crude hydroalcoholic extract of *Aster squamatus* leaves may be beneficial in the treatment of gastric lesions. Further studies to identify the active substances and elucidate the mechanism of action are recommended.

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