Latin American Journal of Pharmacy
(formerly *Acta Farmacéutica Bonaerense)*
Lat. Am. J. Pharm. **26** (6): 889-92 (2007)

Comunicaciones breves

Recibido el 23 de junio de 2007
Aceptado el 11 de agosto de 2007

Butanolic Extract of *Aster squamatus* Aerial Parts is the Active Fraction responsible to the Antiulcer and Gastric Acid Antisecretory Effects

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SUMMARY. ***Aster squamatus*** **is widespread used as antidiarrhoeic, antineoplasic and cicatrizing, and previous studies have revelead an antiulcer effect of crude hydroalcoholic extract of this plant. This led us to determine the active fraction(s) of this extract for antiulcer activity using the ethanol- induced ulcer model. The BuOH-precipitated part from the crude hydroalcoholic extract of aerial parts was determined to be the active fraction. This fraction was also effective to inhibit gastric acid secretion. Further studies should be conducted with the active fraction trying to elucidate the active principle.**

RESUMEN. "El Extracto Butanólico de las Partes Aéreas de *Aster squamatus* es la Fracción Activa Responsable de los Efectos Antiulcerogénico y Antisecretor de Ácido Gástrico". *Aster squamatus* es usado popularmente como antidiarreico, antineoplásico y cicatrizante. Los estudios anteriores demostraron un efecto antiulcerogénico del extracto hidroalcohólico crudo de la planta. Esto nos condujo a determinar las fracciones activas de este extracto para la actividad anti-ulcerogénica usando el modelo de úlcera inducido por etanol. Se determinó que la fracción BuOH del extracto hidroalcohólico crudo de las piezas aéreas es la responsable de la actividad anti-ulcerogénica. Esta fracción es también eficaz para inhibir la secreción ácida gástrica. Estudios posteriores deben ser llevados a cabo con esta fracción para intentar poner en evidencia el principio activo responsable.

INTRODUCTION

Aster squamatus (Spreng.) Hieron. (Asteraceae) is a perennial herb and it possesses worldwide distribution [1]. In Southern Brazil, is known as "erva-milagrosa" or "zé-da-silva" and it is traditionally used as antidiarrhoeic, antineoplasic and cicatrizing [2]. Ethanolic and aqueous crude extracts of leaves, stalks and roots of *A. squamatus* imply low acute toxicity [3], and the use of infusions of the leaves for 30 days has induced only minor changes on some serum biochemical parameters [4]. Phytochemical screening of *A. squamatus* suggested the presence of steroids, terpenes, flavonoids, phenols, aminogroups, saponins, and tannins, and the infusions of leaves, stalks and roots significantly reduced gastrointestinal propulsion [5]. Preliminary studies performed by Ghedini *et al.* [6] demonstrated antiulcer activity of hydroalcoholic extract of *A. squamatus* leaves on gastric ulcer induced by ethanol, indomethacin and stress. Therefore, the aim of this study was to investigate antiulcer activity of the crude hydroalcoholic extract (CHE) of the aerial parts (stem, leaves and fruits) and to determine the active fraction(s) of this extract. Starting from the active fraction(s), our goal was to verify the activity of this extract over the gastric acid secretion.

MATERIAL AND METHODS

Plant material

The plant *(A. squamatus)* was collected in Santa Maria, Southern Brazil, 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 aerial parts (stem, leaves and fruits) were maintained in a ventilated oven (40 °C) for drying and stabilization. The material was further pulverized in a Willye mill.

KEY WORDS: Antiulcer activity, *Aster squamatus*, Gastric secretion.
PALABRAS CLAVE: Aster squamatus, Actividad Anti-úlcera. Secreción gástrica.

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Crude hydroalcoholic extract preparation

To prepare the CHE, 50 g of aerial parts were immersed in 500 mL of an ethanol-water mixture (70-30%), for 48-72 h, with occasional stirring. The obtained extract was filtered and concentrated in a Fisaton 802D waterbath at 40 °C. Then, the concentrated extract was maintained in an oven at the same temperature until the weight remained constant to determine yield.

Fractionated extracts preparation

The crude hydroalcoholic extract (150 g) was redissolved in water (250 mL) and extracted with n-hexane (4 x 500 mL) at room temperature. After the removal of hexane fraction, the remaining aqueous extract was eluated with chloroform ($CHCl_3$) (4 x 500 mL). Therefore, after the removal of chloroform fraction, the remaining aqueous extract was eluated with ethyl acetate (EtOAc) (4 x 500 mL). After removal of EtOAc fraction, the remaining aqueous layer was extracted with n-butanol (BuOH) (4 x 500 mL). Then, after the removal BuOH fraction, each of the fractions separately, except the final aqueous portion, was evaporated to dryness under reduced pressure to give a hexane extract (HEX) (2.31 g viscous liquid), a chloroform extract (CHL) (2.45 g viscous liquid), an ethyl acetate extract (EtOAc) (9.13 g), and a n-butanol extract (BUT) (38.97 g). The final aqueous layer was despised (Fig. 1).

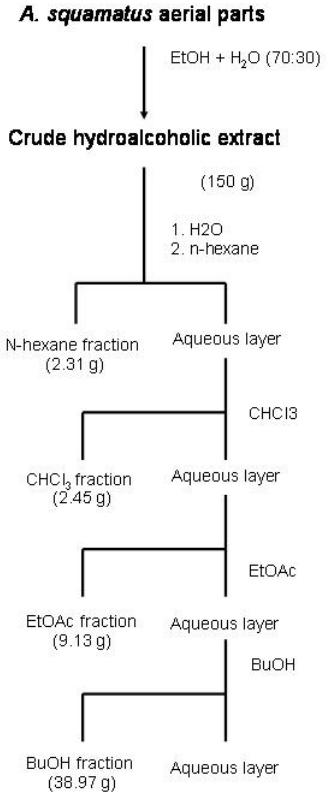

Figure 1. Extraction and fractionation chart of *A. squamatus* aerial parts by solvent extraction.

Pharmacological experiments

Ulceration induced by ethanol

The procedures for ethanol-induced ulcers were an adaptation of the method of Robert *et al.* [7]. After a 24 h fast, male Wistar rats weighing 200-300g (groups of 6 animals) received 250 and 500 mg/kg CHE (0.5 mL/ 100g) through gavage. Starting from the doses of CHE, the doses of the fractional extracts were calculated being taken into consideration the percentage of revenue obtained in each fraction, respectively (4 and 8 mg/kg HEX; 4 and 8 mg/kg CHL; 15 and 30 mg/kg EA; 65 and 130 mg/kg BUT). Control animals were similarly treated with vehicle (distilled water) only. Sixty minutes after this procedure, each 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 computed 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). For this purpose, were evaluated the discoloration of mucosa, the loss of pleats, the presence of petechias or edema, the hemorrhages and the mucus loss. NU was ascertained by direct count of lesions with area size equal to 1 mm or smaller. When larger, the lesions were quantified considering 1,5 points for each mm.

Effects on gastric acid secretion in pylorus-ligated rats

Six male Wistar rats weighing 200-300g were used in each group. Under ether anesthesia, the pyloric sphincter was ligated surgically, as described by Vissher *et al.* [8]. BUT (65 and 130 mg/kg), ranitidine (50 mg/kg) or vehicle (distilled water) were immediately administered intraduodenally and the incision was closed. Sixty minutes after this procedure, bethanecol (10 mg/kg) or histamine (20 mg/kg) were administered subcutaneously. Three hours later the animals were killed, the stomachs were excised, opened along the smaller curvature and the luminal contents were collected and centrifuged for 30 min at 1500 rpm. The volume (mL) and

the gastric pH value were measured. An aliquot (1 mL) of each sample was titrated against 0.01 N NaOH using the phenolphthalein reagent as an indicator. The total acid output was expressed as microequivalents of H^+ per litre per 4 h (mEq[H^+]/L/4h).

Statistical analysis of data

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

RESULTS AND DISCUSSION

Crude hydroalcoholic extract (CHE) and fractions which were obtained from the CHE of *A. squamatus* aerial parts, by successive solvent extractions (HEX, $CHCl_3$, EtOAc, and BuOH) were orally administered to rats and their effects were tested against ethanol-induced ulcer model. As shown in Table 1, the anti-ulcerogenic activity of the BuOH extract was found to be the unique active fraction, similarly to the anti-ulcerogenic activity observed in CHE. The HEX, $CHCl_3$, and EtOAc extracts had no demonstrated effects.

The following step was to verify the activity of the active fraction toward the gastric acid secretion. The BuOH extract (130 mg/kg) inhibited significantly gastric acid secretion in pylorus-ligated rats when stimulated with histamine subcutaneously (Table 2). The effects were not significantly when the secretion was stimulated subcutaneously with the bethanechol (Table 3).

Treatment	N	Lesion Index	Number of Ulcers
Control (vehicle)	6	7.57 ± 0.64	115.57 ± 13.58
CHE 250 mg/kg	6	3.85 ± 0.34*	0.42 ± 0.29*
CHE 500 mg/kg	6	3.28 ± 0.28*	-*
HEX 4 mg/kg	6	8.71 ± 0.74	134.64 ± 10.79
HEX 8 mg/kg	6	8.20 ± 0.73	132.00 ± 18.89
CHL 4 mg/kg	6	6.50 ± 0.42	97.08 ± 9.41
CHL 8 mg/kg	6	9.40 ± 0.60	125.90 ± 7.80
EA 15 mg/kg	6	7.42 ± 0.71	116.64 ± 8.35
EA 30 mg/kg	6	7.42 ± 0.99	88.92 ± 32.46
BUT 65 mg/kg	6	3.28 ± 0.28*	0.14 ± 0.14*
BUT 130 mg/kg	6	2.00 ± 0.63*	0.16 ± 0.16*

Table 1. Effects of the aerial parts crude hydroalcoholic extract and fractionated extracts of *A. squamatus* on ethanol-induced gastric lesions in rats. Each value represents the mean ± SEM. * Significantly different from control P<0.05.

The phytochemical screening data of *A. squamatus* have revealed among other constituents of its extract the presence of saponins [5]. It is known that triterpenoid saponins are effective in the treatment of ulcers [9]. In other species of the genus Aster, *Aster batangensis,* were isolated triterpenoid saponins of the n-butanol fraction [10]. It was suggested that the anti-ulcerogenic action may be related, at least partly, to this constituent.

Treatment	Dose (mg/kg)	N	Total gastric contents (mL)	Gastric pH	Gastric acidity (mEq[H^+]/L/4h)
Control (vehicle		6	8.93 ± 1.31	1.97 ± 0.30	58.05 ± 8.37
Ranitidine	50	6	4.93 ± 0.77*	4.83 ± 1.06*	19.00 ± 5.82*
BUT ext	65	6	7.43 ± 1.18	2.36 ± 0.47	60.55 ± 12.89
BUT ext	130	6	4.83 ± 0.21*	4.00 ± 0.50*	22.76 ± 3.21*

Table 2. Effects of the aerial parts butanolic extract of *A. squamatus* on gastric secretion induced by histamine in rats. Each value represents the mean ± SEM. Significantly different from control P<0.05.

Treatment	Dose (mg/kg)	N	Total gastric contents (mL)	Gastric pH	Gastric acidity (mEq[H^+]/L/4h)
Control (vehicle)		6	12.66 ± 0.18	1.52 ± 0.07	48.18 ± 2.98
Ranitidine	50	6	9.00 ± 0.99*	5.65 ± 0.77*	8.61 ± 3.64*
BUT ext	65	6	10.33 ± 0.38	1.51 ± 0.08	58.70 ± 6.50
BUT ext	130	6	9.71 ± 0.65	1.57 ± 0.04	49.23 ± 2.45

Table 3. Effects of the aerial parts butanolic extract of *A. squamatus* on gastric secretion induced by bethanechol in rats. Each value represents the mean ± SEM. * Significantly different from control P<0.05.

BuOH is a solvent of higher polarity and it extracts flavonoids, tannins, saponins among others chemical substances [11]. Several flavonoids (squamatin, ternatin, ramnetim, kaempferol, baicalein, luteolin-7-methyl-ether, and quercetin) were isolated from the flowers of *A. squamatus* from Egypt [12]. Quercetin is known as an anti-ulcerogenic [13] and it inhibits gastric acid production [14]. These data suggest that flavonoids present in *A. squamatus* might be the active principles of the antiulcer and antisecretory activities shown by the BuOH extract.

It was suggested that its possible mechanism of action may be due to the competitive inhibition the acetylcholine response, since the BuOH extract inhibits the secretion gastric acid when stimulated by histamine (agonist of the histamine receptor) and shows no effect when stimulated by bethanechol (agonist of the muscarinic receptor). These evidences were confirmed with studies performed by Porto [15], where the BuOH extract of leaves of *A. squamatus* decreased the gastrointestinal propulsion of rats via a competitive anticholinergic mechanism.

In conclusion, the BuOH extract of aerial parts of *A. squamatus* possess the active principle(s) that inhibit the gastric acid secretion and protect against the gastric mucosal damage induced by ethanol. The antiulcer effect is due, at least partly, to the presence of flavonoids, since they are related to antiulcer and antisecretory effects, however, the involvement of other compounds in this extract, e.g. saponins, should be considered. It is suggested that the mechanism responsible for the antisecretory activity is the competitive anticholinergic action. Further studies are required to isolate and to identify the active substance(s) and to confirm the mechanism of action of the antiulcerogenic and antisecretory effects of *A. squamatus.*

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