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Preliminary Anti-ulcerogenic and Chemical Analysis of the Aerial Parts of *Trixis divaricata* Sprengel

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SUMMARY. The hydro alcoholic extract of the aerial parts of *Trixis divaricata* Sprengel (HET), given orally at a dose of 1000 mg.Kg^{-1}, showed significant anti-ulcerogenic activity in a model of ulcer induced by indomethacin and absolute alcohol in rats. Flavonoids e tannins were identified during the phytochemical screening of the hydro alcoholic extract.

RESUMEN. "Análisis preliminar antiulcerogénico y químico de las partes aéreas de *Trixis divaricata* Sprengel". El extracto hidroalcohólico de las partes aéreas de la *Trixis divaricata* Sprengel (EHT) administrado oralmente en la dosis de 1000 mg.Kg^{-1}, reveló significativa actividad antiulcerogénica en modelos de úlcera inducida por la indometacina y alcohol etílico en ratas. Flavonoides y taninos fueron identificados en la realización del *"Screening"* fitoquímico del extracto hidroalcohólico.

INTRODUCTION

Trixis divaricata Sprengel, belonging to the *Asteraceae* Dumortier family, is a voluble bushy creeper with dense pubescent woody branches. The leaves are pubescent, sessile, lanceolated and acuminate, having a narrow auriculated base and up to 15 cm in length by 4.5 cm in width. The dorsal face is light-colored and tormented, presenting chapters in wide panicules. It is frequently found all over South America, and in Brazil it is mainly found on the edges of forests (Mantiqueira Mountain Range) and in clearings [1]. Apart from the typical variety: *Trixis divaricata* Sprengel var. *divaricata;* five other varieties exist in Brazil: *Trixis divaricata* Sprengel var. *exauriculata* DC; *Trixis divaricata* Sprengel var. *discolor* Griseb; *Trixis divaricata* Sprengel var. *sprengeliana* Baker; *Trixis divaricata* Sprengel var. *odoratissima* Baker and *Trixis divaricata* Sprengel var. *cladoptera* Baker [1]. This plant species and its varieties are popularly known in Brazil as "carvalhinha, celidônea, erva-andorinha, erva-de-mulher, erva-lanceta, guiné, solidônia and raiz-de-cobra" [1-4]. The infusion of the aerial parts of this plant is used in folk medicine of in various regions of Brazil topically for treatment of inflammatory processes of the ocular conjunctiva [1-4], skin wounds and uterine hemorrhages [1]. Bibliographical survey showed that few phytochemical and biological investigations have so far been carried out on this species. Recently, Aranha *et al.* [5] related the anti-inflammatory effect of the crude evaporated extract of this species in animal models using rats (rat paw oedema induced by carrageenin) and mice (ear oedema induced by croton oil). As traditional non-steroidal anti-inflammatory drugs (NSAIDs) increase the risk of clinically important upper gastrointestinal ulcers and bleeding about fourfold [6], the search for alternative therapies devoid of deleterious effects on the integrity gastric mucosa makes the research of natural products with simultaneous anti-inflammatory activity and anti-ulcerogenic activity attractive. The aim of the present paper was to study the anti-ulcerogenic effect of a

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PALABRAS CLAVE: Actividad antiulcerogenica, Alcohol etilico, Indometacina, *Trixis divaricata* Sprengel.

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sample of *Trixis divaricata* Sprengel from the city of Espírito Santo do Dourado, Minas Gerais, Brazil, in experimental models of ulcer induced by indomethacin and absolute alcohol in rats, as well as its chemical profiles.

MATERIAL AND METHODS

Plant material

The aerial parts of *Trixis divaricata* Sprengel were colleted in Espírito Santo do Dourado, Minas Gerais, Brazil, in November 2001 and identified by the Botanist, Prof. Dr. Fernando de Oliveira (São Francisco University of Bragança Paulista). Voucher specimens (No. Vell 842) have been deposited in the Frei Vellloso herbarium, at São Francisco University of Bragança Paulista.

Preparation of Trixis divaricta Sprengel extract

The standardized dried and pulverized leaves (250 g) and stems (500 g) of *Trixis divaricata* Sprengel, were successively extracted in a percolator with 70% ethanol (3 x 2.5 L) as described by Brazilian Pharmacopoeia II edition [7]. Solvents were removed under reduced pressure using a rotary evaporator to obtain the dried extract (23.9 g), resulting in a yield of 3.18% *(w/w)*, after determining the weight of the residue (g.100 g^{-1} of leaves, inflorescences and stems). The dry extract was stored at –20 °C until use in the pharmacological experiments. The dried extract was suspended in aqueous solution containing 12% of Tween 80, *v/v*, for administration to animals, at the appropriate concentrations for pharmacological tests. In this paper, all doses are expressed in terms of dried extract (mg.Kg^{-1} of body weight).

Phytochemical screening

Qualitative chemical analyses of the crude fluid extract of *Trixis divaricata* Sprengel were carried out for the identification of flavonoids (aluminum chloride, ferric chloride, sodium hydroxide, oxalic-boride and Shinoda reactions) and tannins (reactions with glacial acetic acid, basic lead acetate, ferric chloride, ammonium molibidate and a solution of alkaloids in water) following the usual methods [8].

Animals

Albino Wistar rats (200-250 g) were used in this study. The animals were housed in plastic cages (groups of six rats/cage) in a room with controlled temperature (22 ± 2 °C) under a 12 h light/dark cycle with access to standard certified rodent diet and water "*ad libitum*".

Indomethacin induced ulcer

Male Wistar rats, fasted for 24 h, with free access to water, were divided in at least three groups according to the respective treatment employed (12 % solution of Tween 80 in water, cimetidine 100 mg.Kg^{-1} and *Trixis divaricata* 1000 mg.Kg^{-1}). After 30 min of oral treatment, indomethacin (30 mg.Kg^{-1}) was administrated to all groups of animals, according to methodology described by Morimoto *et al.* [9] After 4 h, the animals were sacrificed, their stomachs were removed, and opened along the greater curvature. The ulcerative lesion index of each animal was calculated by summing the following values, according to methodology described by Gamberini *et al.* [10]:

Loss of normal morphology	1 point
Discoloring of mucous membrane	1 point
Mucous edema	1 point
Hemorrhages	1 point
Petechial points (until 9)	2 points
Petechial points (> 10)	3 points
Ulcers up to 1 mm	*n x 2 points
Ulcers > 1 mm	*n x 3 points

* number of ulcers found

The formula used for the calculation of the percentage of ulceration inhibition in the groups treated with indomethacin (30 mg.Kg^{-1}) is described below:

$$\% \text{ Ulceration inhibition} = \frac{\textbf{ILU} \text{ of the negative control}^{1} - \textbf{ILU} \text{ of the test}^{2 \text{ or } 3} \times 100}{\textbf{ILU} \text{ of the negative control}^{1}}$$

where 1 corresponds to the negative control, a 12% solution of Tween 80 in water (10 mL.Kg^{-1}), 2 is cimetidine (100 mg. Kg^{-1}), and 3 *Trixis divaricata* Sprengel (1000 mg. Kg^{-1}).

Absolute ethanol induced ulcer

Male Wistar rats (200-250 g), fasted for 24 h, with free access to water, were divided in groups according to the respective treatment

(12% solution Tween 80 in water, carbenoxolone 200 mg.Kg^{-1} and *Trixis divaricata* 1000 mg.Kg^{-1}). After 30 min of oral treatment, each animal received 1 mL of absolute ethanol orally, according to methodology described by Robert [11]. After a lapse of 1 h, the animals were sacrificed and their stomachs were removed and opened along the greater curvature. The ulcerative lesion index was determined by methodology described by Gamberini *et al.* [10].

The formula used for the calculation of the percentage of ulceration inhibition in the groups treated with absolute ethanol (1 mL) followed the same line as the one used for indomethacin.

Statistical analysis

Results of gastric lesion are presented in the form of mean ± standard error mean (S.E.M.). The percentage of protection was calculated by considering the difference in ulcerative lesion index (ULI) between rats treated with indomethacin, ethyl alcohol and controls. A 100% protection indicates that there was complete inhibition of indomethacin and ethyl alcohol induced gastric lesions, while a 0% protection indicates there was no reduction in the indomethacin and ethyl alcohol induced gastric lesions. Dunnett´s multiple comparison test was used to statistically evaluate the results. *P* values of 0.05 or less were considered as indicative of significance.

RESULTS AND DISCUSSION

In the present study, two experimental models for investigation of the anti-ulcerogenic effects of the hydro alcoholic extract of *Trixis divaricata* Sprengel were used. The results of these tests demonstrated that extract had the capacity of protecting the gastric mucosa of rats submitted to acute treatments with indomethacin and ethanol, respectively. The first experimental model of ulcer in rats used indomethacin, a non-steroidal anti-inflammatory drug (NSAIDs), an irreversible inhibitor of clooxigenase [12], and enzyme critically involved in the protection of gastric cells through the synthesis of endogenous prostaglandins. Among other effects of prostaglandins on gastric mucosal protection and healing; the increase and/or maintenance of mucosal blood flow; the stimulation of mucus secretion, the inhibition of neutrophil adherence and activation, and the ability to protect the stomach against ulcerogenic agents must be highlighted. Suppression of their synthesis increases mucosal susceptibility to damage (suppression of synthesis by NSAIDs) [6]. Fig. 1 shows the results of the anti-ulcerogenic effect of orally administered *Trixis divaricata* extract on indomethacin induced ulcer model. At dose of 1000 mg.Kg^{-1}, HET produced a strong anti-ulcerogenic effect and caused a significant reduction in ULI (74.3%; $p < 0.001$). In this model, the standard drug, cimetidine (100 mg.Kg^{-1}, *per os*), an antagonist of the H_2 receptors present in the parietal cells responsible for gastric acid secretion [9], produced a significant anti-ulcerogenic effect, inhibiting the development of phenomena in percentages of 71.6% ($p < 0.001$). Compared to the efficacy of reference anti-H_2 drug (cimetidine), the inhibition of ulceration after indomethacin injection was similar to the one induced by the HET (1000 mg.Kg^{-1}).

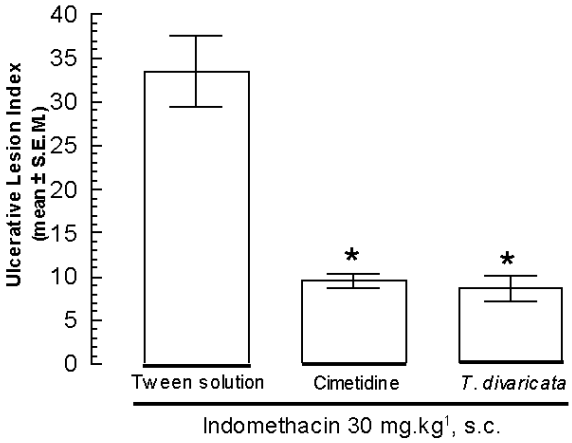

Figure 1. Effect of oral administration of aqueous solution containing 12% of Tween 80 (10 mL.Kg^{-1}), cimetidine (100 mg.Kg^{-1}) and hydro alcoholic extract of *Trixis divaricata* Sprengel (1000 mg.Kg^{-1}), in indomethacin induced ulcer model (ANOVA $F_{(2.26)}$: 30.7, $P < 0.001$. Dunnett's test: $^*P < 0.001$).

Ethanol-induced gastric mucous damage can be easily produced by the generation of exogenous and endogenous active oxygen and free radicals [13]. The process of lipid peroxidation is mediated by the interaction of hydroxyl radicals with the cell membrane; subsequently producing lipid-derivate free radicals such as conjugated dynes and lipid hydro-peroxides. Theses radicals are known to be extremely reactive products that cause oxidative damage. Ethanol increases superoxide anion and hydroxyl radical production and lipid peroxidation in the gastric mucosa [14]. Ethanol treatment induces intracellular oxidative stress and produces mitochondrial permeability transition and mitochondrial depolarization, which precedes cell death in gastric

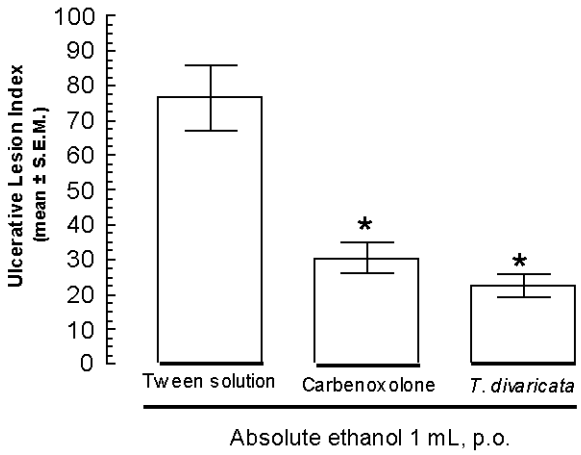

Figure 2. Effect of oral administration of aqueous solution containing 12% of Tween 80 (10 mL.Kg^{-1}), carbenoxolone (200 mg.Kg^{-1}) and hydro alcoholic extract of *Trixis divaricata* Sprengel (1000 mg.Kg^{-1}), in ethanol induced ulcer model in rats. (ANOVA $F_{(2.26)}$: 21.2, $P < 0.001$. Dunnett's test: *$P < 0.001$).

mucous cells and is involved in the formation of free radicals generated extracellularly and/or intracellularly [15].

In the assay of ethanol-induced gastric ulcer, carbenoxolone was used as a positive control. This compound is the sodium salt of the hemisuccinate of glycirinic acid, obtained from the roots and rhizomes of the licorice plant *(Glycyrrhiza glabra* L.) [16]. This natural substance elevates the levels of endogenous prostaglandins, a humoral factor involved in the cellular protection of the gastric mucous membrane [17] through the inhibition of the enzymes; 15-hydroxi-PG-dehydrogenase and Δ^{13}-PG-redutase, responsible for the catabolism of these autacoids [18]. Since prostaglandins are responsible for the production and secretion of gastric mucus which, among other properties, exerts antioxidative and radical scavenging activities, they prevent damages to the mucosa decurrent from lipid peroxidation promoted by the reactive oxygen forms and metabolites generated by ethanol [13]. As shown in Fig. 2, *Trixis divaricata* extract at dose of 1000 mg.Kg^{-1} significantly prevented of the ulcerogenic phenomena induced by the oral administration of absolute ethanol, reducing the ULI in 70.5% ($P < 0.001$), whereas the *Trixis divaricata* extract showed a lower value of ULI when compared to the positive control carbenoxolone (60.2%, $P < 0.001$).

Flavonoids and tannins were detected in extract of *Trixis divaricata* according to the phytochemical screening (Table 1). Flavonoids and tannins are groups with a wide range of biological effects, including anti-ulcer activity [17]. This activity has been described in several studies where indomethacin was used as an ulcer inducing agent in pharmacological trials with extracts containing flavonoids [19,20] and tannins [21,22]. Furthermore, flavonoids [20,23-25] and tannins [26] have also been shown to be efficient in preventing gastric damage induced by the oral administration of absolute ethanol in rats.

Finally, it should be noted that substances such as flavonoids and many others, that possess both anti-inflammatory and anti-ulcerogenic activities are of particular therapeutic importance as most of the anti-inflammatory drugs used in modern medicine are ulcerogenic.

	Reactions				
	Aluminum chloride	Ferric chloride	Sodium hydroxide	Oxalic-Boride	Shinoda
Flavonoids	+	+	+	+	+
	Glacial acetic acid	Lead acetate	Ferric chloride	Ammonium molibidate	Alkaloid solution
Tannins	+	+	+	+	+

(+) Positive qualitative reactions for the presence of the chemical constituents investigated.

Table 1. Results of the qualitative chemical analyses used to verify the presence of flavonoids and tannins in of the extract of *Trixis divaricata* Sprengel.

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