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Antiulcerogenic potential of *Salvia officinalis* L. extract in rats

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ABSTRACT

The objective of this paper was to evaluate the antiulcer activity of a crude ethanol extract of *Salvia officinalis L.* in a gastric lesion induction model. Male Wistars rats were housed in groups of five and fasted 12 hours before the experiment, receiving only water *ad libitum*. The experimental groups received oral doses of 50, 75, 100 and 150 µg/kg of the extract and the negative and positive control groups received distilled water and omeprazol (30mg/kg), respectively. One hour after each respective oral dose, the ulcer was induced with absolute ethanol. a further hour after induction, the animals were euthanized by CO_2 camera, the stomachs removed and opened along the greater curvature. Data was statistically analysed using analysis of variance (ANOVA) followed by the Tukey method ($p<0.05$). The groups treated with *Salvia officinalis* L. extract at doses of 100 and 150 µg/kg showed significant changes in the four parameters evaluated. By reducing the total lesion area, a significant increase in the cure ratio was observed, suggesting a gastroprotective effect of the extract. This data demonstrates the potential use of *Salvia officinalis* L. extract for effective treatment of injuries caused by absolute ethanol.

INTRODUCTION

Salvia officinalis L. is a genus belonging to the family Lamiaceae and has been used as a medicinal plant against a variety of diseases, including gastric disorders and inflammatory process (Alonso, 2004). Extracts of various species of *S. officinalis* L. have been tested for various biological activities such as antimicrobial, anti-inflammatory, antioxidant, spasmolytic and cholinergic and the mechanisms involved have been partially described (Cuvelier *et al.*, 1994; Baracevic and Bartol, 2000; Zupko *et al.*, 2001; Capasso *et al.*, 2004; Ren *et al.*, 2004; Lima *et al.*, 2007). Furthermore, the effectiveness of the *S. officinalis* L. extract in Alzheimer's disease has been reported (Akhondzadeh *et al.*, 2003). In Brazil, *S. officinalis* L. is commonly used for the treatment of gastrointestinal disorders, dyspepsia, liver problem, diabetes, constipation, as well as skin, mouth and gum infections (Lorenzi and Mattos, 2002). Presently, a high incidence of gastrointestinal lesions are being attributed to peptic ulcers, reflux and gastric-related factors of modern life. Consequently, such conditions have become a major focus of experimental investigation with the aim of developing new effective therapeutic agents.

Various vegetable components such as tannins, flavonoids and terpenoids, have shown significant antiulcerogenic activity (Borrelli and Izzo, 2000; Rodriguez *et al.*, 2004; Marques *et al.*, 2006), demonstrating the potential of herbal remedies as an alternative treatment for gastric ulcers. Recent studies have revealed the gastroprotective activity of *S. officinalis* L. against ethanol-induced ulcers, via both free radical scavenging and inhibition of H^+K^+-ATPase pump (Mayer *et al.*, 2009). In addition, the potential therapeutic effect of oleanolic acid for the treatment of acetic acid-induced ulcers in rats has also been demonstrated (Rodriguez *et al.*, 2003).

It has been well documented that the antioxidant properties of some plants are directly related to both gastroprotective (Kahraman *et al.*, 2003) and cytoprotective activities (Mahakunakorn *et al.*, 2003). It was suggested that flavonoids may be the main compounds responsible for the antiulcerogenic activity of plants (Havsteen, 2002). The antioxidant activity of *S. officinalis* L. has been described in several papers (Cuvelier *et al.*, 1994; Lu and Foo, 2001; Tepe, 2008).

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In this sense, the main objective of this work was to evaluate the antiulcerogenic activity of crude ethanol extract of *S. officinalis* L. on model gastric lesion induction with absolute ethanol in Wistar rats.

MATERIAL ANS METHODS

Plant materials and extraction

Leaves of *S. officinalis* L. were collected in Erechim in march 2007 (latitude 27° 38'3" south, longitude 52° 16'26" west, altitude 783m) and identified by Zanin EM. A specimen was deposited at the HERBARA-Herbarium Balduíno Rambo of URI-Campus Erechim under reference number 10013. The leaves were macerated with ethyl alcohol and the obtained solution was evaporated on a rotary evaporator at 35°C to minimize degradation of the extract components. Finally, the extract was dried under air circulation (35°C) for 2 days.

Animals

Thirty adult male Wistar rats (3 month old, 180-220g) were obtained from the Laboratory of Animal Experimentation at URI-Campus Erechim, Brazil.

The animals were kept in a facility under controlled humidity (50±5%) and temperature (22±2°C) with 12h dark/12h light cycles with food and water *ad libitum*. Twelve hours prior to the experiment they were transferred to the laboratory and only given water *ad libitum*. All procedures used in the present study comply with the animal care guidelines outlined by the URI Ethics Committee on the use of animals.

Anti-ulcer activity

Following 12h of fasting, the rats were randomly divided into six groups of five animals. The first group was given 1 mL of vehicle (distilled water), and the second group was treated with omeprazole (30 mg/kg). The remaining groups received 50, 75, 100 and 150 µg/kg of crude ethanolic extract of *S. officinalis* L., each of the treatments being administered orally. One hour after treatment, each rat received 1 mL of 99.5% ethanol to induce gastric ulcer.

The animals were euthanized by CO_2 camera one hour after induction, the stomachs removed and opened along the greater curvature. The stomachs were rinsed with water to remove gastric contents and blood clots, for subsequent scanning. The images obtained were analyzed using specific "EARP" software to measure each lesion point.

The ulcers were classified as level I (ulcer area<1mm^2), level II (ulcer area 1-3mm^2) or level III (ulcer area>3mm^2). The parameters were determined, following the protocol described by Andrade et al. (2006):

(1) Ulcerative lesion index (ULI): 1x (number of ulcers level I) + 2x (number of ulcers of level II) + 3x (number of ulcers level III);

(2) Curative ratio: %C= 100-(ULI treated x 100/ ULI control)

(3) Total area of lesion

(4) Percentage of lesion area in relation to total stomach area

Statistical analysis

The values were expressed as the mean value ± standard error. Statistical evaluation of the resultant data was performed using one-way analysis of variance (ANOVA) followed by the Tukey method. Values of $p<0.05$ were regarded as significant in GraphPad Prism 5 software.

RESULTS AND DISCUSSION

Gastric ulcer disease is defined as an imbalance between mucosal defense factors (bicarbonate, mucin, prostaglandin, nitric oxide, other peptides and growth factors) and aggressive factors (acid and pepsin) (Goodman and Gilman, 2005). It has been suggested that the protective mechanisms of *Salvia officinalis* L. against gastric stomach lesions are due to its antioxidant activity and the role it plays in gastric juice reduction (Mayer *et al.*, 2009).

The results obtained regarding the evaluation of the antiulcer activity of the crude ethanolic extract of *S. officinalis* L. are represented in Table 1.

The total area of lesion (mm^2) was reduced in the groups receiving the extract at doses of 50 ($p<0.05$), 75 ($p<0.05$), 100 ($p<0.01$), and 150µg/kg ($p<0.05$). This was also observed for the positive control (treated with omeprazole ($p<0.05$)) in comparison to the negative control (treated with distilled water). Regarding the percentage of lesions, only doses of 100 ($p<0.05$) and 150 µg/kg ($p<0.05$) were significantly lower than the negative control group. All four test doses and also the positive control were able to significantly reduce the ulcerative lesion index compared to the negative control group. In the calculation of curative ratio, in addition to the positive control group treated with omeprazole ($p<0.01$), a significant increase in the groups treated with *S. officinalis* L. extract was observed at doses of 50 ($p<0.05$), 75 ($p<0.05$), 100 ($p<0.001$) and 150µg/kg ($p<0.001$).

There are several drugs available for the treatment of ulcers, however, many of these drugs are quite expensive and are also associated with several side-effects and toxicity issues. Factors such as these have stimulated research into potential herbal remedies as alternative gastroprotective agents (Santin *et al.*, 2010).

Regulation of acid secretion in the stomach is essential in counteracting the effects of ulcer pathogenesis. Such mechanisms are being exploited by existing agents for the treatment of acid-peptic diseases such as proton pump inhibitors and the receptor antagonists histamine H2 (Goodman and Gilman, 2005).

The induction of gastric lesions by drugs (anti-inflammatory nonsteroidal), stress, or ethanol are the three most popular models used to evaluate the antiulcer activity of new investigative drugs. Moreover, these three models have been reported as being most representative of the pathogenic mechanisms associated with gastric ulcers in humans (Lapa *et al.*, 2008). Ethanol is known to cause necrosis via the destruction of the mucus layer, increasing its vascular permeability, leading to induction of a proportion of ulcers independent of gastric acid secretion. Gastric mucosal injury is characterized by hyperemic

and hemorrhagic foci, resulting in a rapid increase in mucosal blood flow (Glavin and Szabo, 1992).

According to Gonzalez et al. (2001), the model of ethanol-induced ulcers results in direct damage to cells of the gastric mucosa, provoking the formation of free radicals and lipid hyperoxidation. Such finds suggest that antioxidant compounds may result in a reduction of ulcer activity in such experimental models.

Carnosic acid and carnosol have demonstrated their ability to act as pro-inflammatory agents, inhibiting polymorphonuclear cells, formation of leukotrienes and generation of reactive oxygen species (Poeckel *et al.*, 2008). Also, the anti-inflammatory activity of leaf extracts of *S. officinalis* L. isolated from various locations were investigated, where inhibition was observed, dependent on the dose and level of paw edema of mice induced with cottonseed oil (Baracevic *et al.*, 2001).

The organic compound, diterpene, is the major constituent of the *S. officinalis* L. extract, which appear to be related to the anti-inflammatory properties of the plant. Additionally, propolis oil has been found to contain the compounds caryophyllene oxide and caryophyllene, both of which have displayed anti-inflammatory activity (Sousa *et al.*, 2006) and different extracts of *S. officinalis* L. cultivated in southern Brazil (Dal Prá *et al.*, 2011) have been shown to contain these compounds. Baracevic et al. (2001) showed that ursolic acid was the main component of different fractions of *S. officinalis* L. chloroform extract.

In a previous study of the constituents of *S. officinalis* L. gastroprotectors (Mayer *et al.*, 2009), oral administration of hidroalcoholic extract (30, 100, 300 and 1000 mg/kg) 1 hour before de induction of gastric lesions with 80% ethanol, decreased the area of lesions with ED_{50}:84.0 (54,8-128.9) mg/kg. Omeprazole, positive control of the test reduced the gastric lesions induced by ethanol in 58%. Among the compounds highlighted as being responsible for the observed activity are phenolic compounds such as flavonoids, which possess antioxidant properties. This study demonstrated that the compound carnosol, is a major contributor to gastroprotective activity.

Our study differs from those performed by Mayer et al. (2009) in some aspects. For instance, Mayer used doses of 30, 100, 300 and 1000 mg/kg of a hydroalcoholic (85% ethanol) preparation of dried leaves of *S. officinalis* L., supplied by a company in Paraná, Brazil. Conversely, the doses used in this study were much lower (50, 75, 100 and 150µg/kg of ethanolic extract), being intermediate studied by the research group in Toxicology URI-Campus Erechim, where the observed anti-inflammatory activity of the extract gross *S. officinalis* L. Dal Prá et al. (2011) verified in their study anti-inflammatory activity of the methanol extract of *S. officinalis* L. dose of 25µg/kg induction of acute peritonitis. Some of the advantages of using lower dose delivery include, lower risk of intoxication and adverse effects, better patient adherence to pharmacological treatment, among others.

Climate difference (temperature, soil) is one factor that may interfere with the development of plants. The respective constituents and properties of each plant will vary according to the region where it is located, which also distinguishes this study from others. In the present study, we observed that in the negative control group (treated with distilled water), the quick development of lesions was observed in the mucosa of the stomach, confirming the necrotizing action of ethanol. This control confirms the validity of this technique for the investigation of potential anti-ulcer agents.

The positive control group (omeprazole) demonstrated a significant reduction in both the total area of stomach mucosa lesions and also ulcerative lesion index, resulting in a significant percentage of healing. These results confirm the effect of omeprazole in inhibiting the Na^+/K^+ pump, previously reported in the literature (Dent *et al.*, 1994; Havelund *et al.*, 1994;Vakil and Fennerty, 2003). Omeprazole has been shown to be unstable under acid conditions, which means it is usually administered in the form of pellets with enteric coating. Since the animals in this study received an oral dose of omeprazole, it is possible that there was some instability of the omeprazole due to the acidic environment of the stomach. This factor may have interfered with the analysis of certain parameters, which if avoided could have lead to a greater cure percentage value or even a greater reduction in the lesion and ulcerative lesion index.

The treated groups receiving oral doses of 100 and 150 µg/kg presented significant changes for the four parameters tested. By decreasing the total lesion area, a quite significant increase in cure rate was observed in these groups, suggesting a gastroprotective effect of crude extract of *S. officinalis* L. Previous studies have concluded that the carnosic acid and its oxidation product carnosol are major constituents of *S. officinalis*, responsible for antioxidation, anti-inflammatory, antiproliferative and neuroprotective properties. Therefore, it is believed that one of the mechanisms of *S. officinalis* L. to protect the stomach against gastric lesions is through its antioxidant activity and the reduction of gastric acid secretion (Poeckel *et al.*, 2008; Mayer *et al.*, 2009).

Table. 1: Effect of the oral administration of omeprazole (30 mg/kg) and the varying doses of crude *S. officinalis* L. ethanolic extract (50, 75, 100 and 150 µg/kg) on ethanol-induced gastric ulcers in rats.

Treatment v.o	Dose (µg/kg)	Total area of lesion (mm^2)	Percentage of lesion	Ulcerative lesion index	Curative ratio (%)
Negative control	---	151,4±31,86	10,24±2,37	45,00±5,41	---
Omeprazole	30 mg/kg	50,76±20,73 [a]	4,33±1,73	23,00±4,12 [a]	55,58±7,97 [b]
Extract	50	109,14±29,96 [a]	8,83±2,83	37,5±4,12 a	30,56±7,63 [a]
	75	35,25±15,31 [a]	3,4±1,35	22,0±4,05 [a]	42,43±11,9 [a]
	100	29,63±29,52 [b]	2,57±2,45 [a]	18,75±7,04 [c]	65,28±13,03 [c]
	150	29,55±9,37 [a]	2,30±0,65 [a]	16,00±4,05 [a]	52,60±11,99 [c]

The results shown are the mean values ± S.E.M. for five rats. Statistical comparison was performed using ANOVA followed by the Tukey method st. [a]p <0,05, [b]p <0,01, [c]p <0,001 in comparison with the negative control group.

CONCLUSION

The data obtained in this study showed that the crude extract of *Salvia officinalis* L. at doses of 100 and 150 µg/kg is capable of significantly reducing injuries caused by absolute ethanol, suggesting a possible anti-ulcerogenic activity in rats. Further studies are required to fully confirm to be so the mechanisms involved can be elucidated in the possible gastroprotective effect of *S. officinalis* L.

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