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Antiulcerogenic Effect of *Securigera securidaca* L. Seed Extract on Various Experimental Gastric Ulcer Models in Rats

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Abstract: *Securigera securidaca* belongs to the family Fabaceae is used in Iranian folk medicine to treat gastric disturbances. The present study was undertaken to evaluate the *Securigera securidaca* seed hydroalcoholic extract (SSE) and its subfractions for their gastroprotective effect in rat. Acute gastric ulceration in rats was produced by oral administration of ethanol (100%; 1 mL/200 g of body weight) or water immersion restraint-stress (5 h, water immersion restraint stress at 20-22°C). Ranitidine (100 mg kg^{-1}, p.o.) was used as the reference antiulcer drug. After ethanol administration, the gastric wall mucus was examined. Chronic gastric ulceration was produced by injection of acetic acid in rat gastric subserosa. The antisecretory effect of the extract and its subfractions (ethyl acetate, chloroform and aqueous fractions) were investigated in pylorus-ligated rats. Administration of SSE significantly inhibited gastric mucosa damage induced by ethanol, water immersion restraint-stress and acetic acid in a dose-dependent manner. In pylorus ligature rats, SSE and its subfractions significantly reduced the basal gastric acid secretion and total acidity; moreover, it inhibited the increase in total acidity induced by carbachol. However, the antisecretory effect of the chloroform fraction was more potent than two other fractions. Administration of SSE did not affect the gastric mucus production. The results obtained in the present study indicate that the SSE has gastroprotective and antisecretory effects on gastric mucosa in rats.

Key words: *Securigera securidaca*, rat, water immersion restraint-stress

INTRODUCTION

Peptic ulcer disease (encompassing gastric ulcer and duodenal ulcer) affect a large portion of the world population and are induced by several factors, including stress, smoking, nutritional deficiencies and ingestion of non-steroidal anti-inflammatory drugs (Nash *et al*., 1994). The pathophysiology of these ulcers involves an imbalance between offensive (acid, pepsin and *Helicobacter pylori*) and defensive factors (mucus, prostaglandin, bicarbonate, nitric oxide and growth factors). Today, there are two main approaches for treating peptic ulcer. The first deals with reducing the production of gastric acid and the second with re-enforcing gastric mucosal protection (Hoogerwerf and Pasricha, 2001; Valle, 2005). There has been a rapid progress in the understanding of the pathogenesis of peptic ulcer. Modern approach to this includes proton pump inhibitors, histamine receptor blockers, drugs affecting the mucosal barrier and prostaglandin analog (Manonmani *et al*., 1995). Development of tolerance and incidence of relapses as well as side effects on clinical evaluation make their efficacy arguable. This has been the basis for the development of new antiulcer drugs, which includes herbal drugs.

It has been shown that the extracts from the seeds of *Securigera securidaca* (Fabaceae) have different activities such as marked chronotropic, diuretic and hypokalaemic activities (Ali *et al*., 1998). It has been reported that an aqueous extract of the seeds reduced blood glucose levels in anaesthetized cats (Nagarajan *et al*., 1982). The phytochemical analysis of the ethanolic and aqueous extracts of *Securigera securidaca* seed indicated the presence of flavonoids, alkaloids, saponins and tannin (Hosseinzadeh *et al*., 2002).

The powder of the seeds of *Securigera securidaca* is used in traditional medicine in South-West of Iran to treat heartburn resulted from gastric reflux. The present study was carried out to evaluate the gastroprotective effect of the hydroalcoholic extract of the seed from *Securigera securidaca* against acute and chronic experimental models of gastric ulcer in rats.

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MATERIALS AND METHODS

Plant material, preparation of hydroalcoholic extract and fractions: *Securigera securidaca* seed were purchased from the local market of Ahwaz and authenticated by Dr. N. Alemzadeh Ansari (Faculty of Agriculture, Ahwaz Chamran University). The seeds powdered and extracted by maceration with 70% ethanol (1:10, w/v) for 72 h. The combined ethanolic extract was filtered through cheese cloth and centrifuged (3500 rpm, 15 min). The supernatant was concentrated and lyophilized for preservation (yield: 11%) which was stored at -4°C until used.

Activity-guided fractionation was carried out by using solvents of increasing polarity to obtain organic and aqueous fractions. Twenty gram of hydroalcoholic extract (SSE) was dissolved in about 50 mL of distilled water. The same volume of the chloroform was added to it with vigorous shaking. The chloroform layers (lower) were collected thrice and evaporated on rotary evaporator to give the chloroform fraction (PDE.$CHCl_3$). The other layer (upper) was again taken into a separating funnel, ethyl acetate was added into it, separated and was also evaporated in rotary evaporator to give the ethyl acetate fraction (PDE. EtAc). The remaining lower layer was collected and evaporated to obtain the aqueous fraction (PDE. Aq) (Gilani *et al*., 2008).

Animals: This study was carried out in Ahwaz Jundishapoor University of Medical Sciences in January 2008. Male Wistar rats weighing 150-180 g were obtained from the animal house of Ahwaz Jundishapoor University of Medical Sciences. Animals were fed on conventional diets and water *ad libitum* and they were maintained under standard conditions of humidity, temperature (20-24°C) and light (12 h light: 12 h dark cycle). The rats were randomly assigned to control and different treatment groups, 6 animals per group. All animal experiments were carried out in accordance with Ahwaz Jundishapoor University of Medical Sciences, Ethical Committee acts.

Induction of acute gastric lesions in rats: In ethanol-induced ulcer model, rats were starved of food but not water for 24 h. Animals were orally pretreated with vehicle (saline, 0.5 mL/100 g b.wt.), SSE (100, 200 and 400 mg kg^{-1}) or ranitidine (100 mg kg^{-1}), 60 or 30 min before receiving ethanol (1 mL 200 g $^{-1}$ of body weight) (Sairam *et al*., 2002). Each test compound (saline, SSE and ranitidine) was given orally in a volume of 0.5 mL/100 g of body weight.

In order to evaluate the degree of gastric mucosal lesions, the length (mm) and width (mm) of hemorrhagic erosions in the gastric mucosa were measured and the surface area of each erosion (mm^2) was calculated using following formula:

Surface area (UI) = Length×width×π/4 (Xing *et al*., 1998)

The total area of each erosion is expressed in terms of ulcer index (mm^2): In the water immersion stress-induced gastric ulcer model, fasted rats (48 h) were orally pretreated with vehicle (saline, 0.5 mL/100 g b.wt.), SSE (100, 200 and 400 mg kg^{-1}) or ranitidine (100 mg kg^{-1}) 60 or 30 min prior to stress exposure. Rats were restrained individually in plexiglass restrainer and immersed up to their xiphoid in a water bath (20-22°C) for 5 h (Gharib Naseri and Mard, 2007). Rats were then removed from restrainer and sacrificed by an overdose of diethyl ether and their stomachs removed and opened along the greater curvature and examined for gastric ulcers. The degree of gastric mucosal injury was expressed as the gastric ulcer index (mm^2) as mentioned in measurement of ethanol-induced gastric mucosal injury.

Induction of chronic gastric lesions in rats: Induction of chronic gastric ulcers was studied according to the method described previously by Twardowschy *et al*. (2008). A solution of 0.05 mL 30% acetic acid was injected under ether anesthesia under the stomach serosa of the animals. After the application of acetic ulcers, the animals were allowed to recover from anesthesia and received only water at the day of operation. Starting on the 2nd day after acetic acid injection, animals were orally treated with vehicle (saline, 0.5 mL/100 g b.wt.), SSE (100, 200 and 400 mg kg^{-1}) or ranitidine (100 mg kg^{-1}) once a day for 5 days. On the day following the last administration the animals were sacrificed, the stomach were removed and the extent of the gastric ulcer was measured.

Determination of gastric acid secretion in rats stimulated with histamine and carbachol: The animals were separated in eleventh groups: (1) control (saline); (2) histamine; (3) histamine plus ranitidine; (4) histamine plus SSE; (5) carbachol; (6) carbachol plus atropine; (7) carbachol plus SSE; (8) SSE chloroform fraction; (9) SSE ethyl acetate fraction; (10) SSE aqueous fraction and (11) SSE. A pylorus ligature (Nguelefack *et al*., 2008) was carefully performed in fasted male rats under anesthesia. Animals received vehicle (saline, 0.5 mL/100 g b.wt.), SSE (100, 200 and 400 mg kg^{-1}), SSE subfraction(s) (200 mg kg^{-1}), ranitidine (100 mg kg^{-1}) orally 30 min prior to pylorus ligation or atropine (1 mg kg^{-1}, s.c.) was administered in the moment of the ligature proceeding. One hour after pylorus ligation, the animals received histamine (20 mg kg^{-1}, s.c.) or carbachol (4 μg kg^{-1}, i.p.) stimulus. Five hours after pylorus ligation, animals were euthanized by deep anesthesia, the stomach was opened and gastric secretion collected. The volume of the gastric juice supernatant was determined and the acidity was determined by an autotitrator pH meter

(PHM 85, Radiometer, Copenhagen, Denmark) to end point pH 7 with 0.01 N NaOH and expressed as μEq H^+/5 h (Twardowschy *et al*., 2008).

Gastric wall mucus determination: The procedure of Perera *et al.* (2001) was used to determine the gastric-wall mucus. After washing with normal saline, the gastric mucus obtained by scraping the mucous was homogenized in 4 mL of distilled water. The weight of mucus (g) was obtained from the difference between the weight of homogenate and the original 4 mL of water.

Materials: Ethanol, diethyl ether, chloroform, ethyl acetate, acetic acid and NaCl were purchased from Merck (Germany) and ranitidine from Tolidaru (Iran).

Statistical analysis: The results are expressed as Mean±SEM (n = number of animals in each group) and statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Student's t-test. p-values of less than 0.05 were considered to indicate a significant difference between means.

RESULTS

Effect of the SSE in acute gastric ulcers in rats: Rat mucosal gastric injury induced by ethanol was reduced dose dependently ($p<0.01$) by SSE (Table 1). Also, the *Securigera securidaca* hydroalcoholic extract reduced the mucosal gastric lesion induced by water immersion restraint-stress test dose dependently ($p<0.01$). Ranitidine (100 mg kg^{-1}, p.o.) protected the animals from ulceration significantly (Table 1).

Effect of the SSE in chronic gastric ulcers induced by acid acetic: The oral administration of SSE reduced the chronic gastric ulcer induced by acetic acid in a dose dependent manner ($p<0.01$) and the treatment with ranitidine (100 mg kg^{-1}, p.o.) reduced the ulceration significantly (Table 1).

Effect of the SSE and its subfraction(s) on gastric acid secretion with or without stimulation: In pylorus ligature rats, pre-treatment with SSE (100, 200 and 400 mg kg^{-1}) reduced the increase in gastric secretion volume and total acidity to 6.1±05.6 mL and 110.42±9.1 μEq. [H+]/5 h; 5.9±0.65 mL and 105.32±5.13 μEq. [H+]/5 h and 5.2±0.36 mL and 58.32±5.11 μEq. [H+]/5 h, respectively from 7.8±0.88 mL and 140.29±17.3 μEq. [H+]/5 h of the control group (saline). SSE subfractions were also significantly decreased the gastric secretion volume and total acidity as compared to control rats ($p<0.01$). Gastric secretion volume and total acidity was increased by histamine (s.c.) to 11.7±0.5 mL and

Table 1: Effect of oral administration of SSE on gastric ulcers in rats

	Gastric ulcer inducer					
	Ethanol		WIRS		Acetic acid	
Treatments	Ulcer index (mm^2)	I (%)	Ulcer index (mm^2)	I (%)	Ulcer index (mm^2)	I (%)
Saline (0.5 mL/100 g b. wt.)	77.31±3.4	0	21.2±2.2	0	38.1±2.5	0
Extract (100 mg kg^{-1})	52.42±6.1*	32	11.2±1.8*	48	30.5±3.8*	20
Extract (200 mg kg^{-1})	16.37±2.1**	79	6.5±1.2**	70	21.3±2.1**	44
Extract (400 mg kg^{-1})	8.12±1.1***	90	4.2±1.1**	81	13.7±1.5**	64
Ranitidine (100 mg kg^{-1})	34.53±4.4**	55	3.2±1.8**	85	10.5±1.8**	77

The results are expressed as Mean±SEM (n = 6); I (%): Inhibition of ulcer formation expressed as percentage. Statistical comparison was performed using ANOVA and followed by t-test. *$p<0.05$, **$p<0.01$ and ***$p<0.001$when compared to control group

Table 2: Effects of SSE and its subfractions on the volume, pH and total acidity of gastric acid secretion in rats with or without stimulation by histamine or carbachol, in the pylorus ligature rats

Treatments	Volume of gastric content	pH	Total acidity (μEq H^+/5 h)
Saline (0.5 mL/100 g b. wt.)	7.80±0.88	3.84±0.11	140.29±17.3
Extract			
100 mg kg^{-1}	6.10±0.56[a]	5.05±0.12[a]	110.42±9.1[a]
200 mg kg^{-1}	5.90±0.65[a]	5.42±0.19[a]	105.32±5.1[a]
400 mg kg^{-1}	5.20±0.36[a]	6.21±0.23[a]	58.32±5.1[a]
SSE subfractions (200 mg kg^{-1})			
Chloroform	5.05±0.08[a]	6.15±0.31[a]	54.61±3.23[a]
Ethyacetate	5.61±0.18[a]	5.86±0.44[a]	74.53±7.77[a]
Aqueous	6.22±0.54[a]	5.18±0.43[a]	114.15±7.34[a]
Hist	11.70±0.54[a]	3.61±0.09[a]	180.28±12.03[a]
Hist + Ran	6.65±0.37[b]	5.14±0.13[b]	123.36±11.07[b]
Hist + SSE (400 mg kg^{-1})	10.94±0.86	3.75±0.06	171.56±13.16
Cch	12.75±0.35[a]	3.41±0.05[a]	195.56±18.72[a]
CCh +Atr	5.49±0.17[c]	6.05±0.12[c]	61.76 ± 3.14[c]
CCh + SSE (400 mg kg^{-1})	7.23±0.45[c]	5.88±0.09[c]	121.73±13.6[c]
Ranitidine (100 mg kg^{-1})	6.20±0.23[a]	5.88±0.09[a]	75.20±4.4[a]

Hist: Histamine 20 mg kg^{-1}, s.c.; Cch: Carbachol, 4 μg kg^{-1}, i.p.; Atr: Atropine, mg kg^{-1}, s.c.; Ran: Ranitidine, 100 mg kg^{-1}, p.o. The results are expressed as Mean±SEM (n = 6). Statistical comparison was performed using ANOVA and followed by t-test. [a]$p<0.05$ when compared with control group; [b]$p<0.05$ when compared with Hist; [c]$p<0.05$ when compared with Cch

Table 3: Effect of oral administration of SSE on gastric mucus content in the ethanol-induced gastric ulcer animals

Treatments	Gastric mucus (g)
Saline (0.5 mL/100 g b. wt.)	0.21±0.08
Extract	
100 mg kg^{-1}, p.o.	0.21±0.05
200 mg kg^{-1}, p.o.	0.22±0.02
400 mg kg^{-1}, p.o.	0.23±0.04
Ranitidine (100 mg kg^{-1}, p.o.)	0.31±0.09**

The results are expressed as Mean±SEM (n = 6). Statistical comparison was performed using ANOVA and followed by t-test. *p<0.05 and **p<0.01 when compared to control group

180.28±12.03 μEq. [H+]/5 h, respectively from 7.8±0.88 mL and 140.29±17.3 μEq. [H+]/5 h of the control group (saline). Gastric secretion volume and total acidity were also increased by carbachol to 12.75±0.3 mL and 195.56±15.72 μEq. [H+]/5 h, respectively from 7.8±0.88 mL and 140.29±17.3 μEq. [H+]/5 h of the control group (saline). In carbachol groups, pre-treatment with SSE (400 mg kg^{-1}) block the increase in gastric secretion volume and total acidity and pre-treatment with atropine (s.c.) also blocked the increase to 5.14±0.17 mL and 112.35±8.35 μEq.[H+]/5 h (Table 2). Also pre-treatment with ranitidine blocked the increase to 6.2±0.23 mL and 75.29±4.44 μEq.[H+]/5 h.

Effect of SSE on gastric wall mucus production: In ethanol-induced gastric ulcer rats, orally administration of SSE at doses 100, 200 and 400 mg kg^{-1} did not affect the gastric mucus secretion (Table 3).

DISCUSSION

The present study showed that the hydroalcoholic extract of *Securigera securidaca* and its subfractions have an effective antisecretory in pylorus ligature rats and anti-ulcer activity against water immersion restraint-, ethanol- and acetic acid-induced gastric ulcers.

The gastroprotective effect of the extract may be related to an antacid effect or cytoprotective properties of the extract. The gastroprotective action against ethanol showed that the effect of extract is mainly mediated by the inhibitory effect of the SSE on gastric acid secretion or maybe due to the inhibition of gastric pepsin secretion. Also, the gastroprotective action of the SSE against ethanol maybe due to the promotion of gastric blood flow, the stimulation of growth factors or the stimulation of the bicarbonate secretion. Administration of the extract did not show any effect on gastric mucus production, therefore, the extract had not the cytoprotective effect.

Phytochemical studies of the seeds of *Securigera securidaca* revealed the presence of flavonoids, saponins, alkaloids and tannin (Hosseinzadeh *et al*., 2002). Flavonoids have antiulcer and gastroprotective activity (Alvarez *et al*., 1999; Reyes *et al*., 1996). Saponins isolated from the seeds of the tea plant [*Camellia sinensis* (L.) O. KUNTZE (Theaceae)] exhibited potent protective effects on ethanol- and indomethacin-induced gastric mucosal lesions in rats (Yoshikawa *et al*., 2005). Since, flavonoids and saponins have been shown to be present in the SSE (Hosseinzadeh *et al*., 2002), it is possible that these constituents may be implicated in the antiulcer activity of the species.

It is possible that the inhibitory effect of SSE in this experimental model is due, at least partly, to the presence of tannins. Tannins were associated to antiulcerogenic activity in other plants (Berenguer *et al*., 2006; Vallet *et al*., 1994).

Another important protective factor is the inhibition of acid secretion (Konturek *et al*., 2005), since when levels of acid overwhelm mucosal defense mechanisms this leads to ulcer formation (Schubert, 2004). In present study, SSE and its subfractions were tested in the pylorus ligature model, where gastric volume and total acidity were reduced during 5 h of pylorus ligature in rats. To verify the mechanisms responsible for the anti-secretory action of SSE, the gastric acid secretion was stimulated with two secretagogues, histamine and carbachol. The animals that received pre-treatment with SSE showed inhibition of the increase in gastric volume and total acidity stimulated by carbachol, however, SSE did not inhibit the stimulation by histamine. It is possible, therefore, that the extract acts via., an anticholinergic mechanism and blocks the gastric acid secretion.

The extract provoked a marked decrease in gastric acid content and increase in pH values. However, the extract had no significant effect on mucus secretion. These finding suggest that the antiulcerogenic effect of SSE mainly mediated through an anti-secretory action but not due to an increase in defensive factors such as mucus secretion.

In conclusion, *Securigera securidaca* seed markedly inhibits acid secretion and the occurrence of lesions in stomach but exact mechanisms are not clear yet. The precise mechanism of action of SSE in protecting rats against induced gastric lesions is unknown. Further studies with isolated compounds are needed to elucidate the active principles and mechanisms involved in this activity.

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