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Research Article

ANTIULCER ACTIVITY OF THE ETHANOL EXTRACT OF LEAVES OF *SESBANIA GRANDIFLORA* (LINN.)

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ABSTRACT

Background & objectives: Sesbania grandiflora has long been used in folk medicine in treatment of diarrhoea, snake bite, malaria, smallpox, fever, scabies; ulcer, and stomach disorders. Therefore, Present study was designed to investigate the antiulcer effect of ethanolic extract of leaves of S. grandiflora (EELSG) using different models of gastric ulceration in rats.

Methods: Acute gastric ulceration in rats was produced by oral administration of various noxious chemicals including aspirin or ethanol or indomethacin. Anti-secretory studies were undertaken using pylorus-ligated technique. Gastric total acid output was estimated in the pylorus ligated rats. Gastric tissue was also examined histologically. EELSG was administered in the dose of 400 mg/kg orally in all experiments. Omeprazole, ranitidine, misoprostol were used as a reference drug. The antiulcer activity was assessed by determining and comparing the ulcer index in the test drug group with that of the vehicle (negative) control group and standard (positive) control group.

Results: EELSG at the dose of 400 mg/kg produced a significant reduction in the ulcer index. EELSG significantly inhibited gastric mucosal damage induced by aspirin, ethanol and indomethacin. In pylorus-ligated Shay rats, EELSG significantly reduced the basal gastric acid secretion. The anti-ulcer effect was further confirmed histologically. The anti-ulcer activity of EELSG was however, less than that of standard drugs.

Interpretation & conclusion: The present finding suggests that protective effect of EELSG might have been mediated by both anti-secretory and cytoprotective mechanisms. Moreover, further insight into the precise mechanism of action is essential to exploit the complete potency of EELSG and increase its usage in contemporary medicine.

Keywords: Antiulcer action, Ethanolic extract, Preventive effect, *Sesbania grandiflora.*

INTRODUCTION

Sesbania grandiflora Miq (Fabaceae), popularly known as "Basna", is a short-lived, quick-growing, soft-wooded tree, 6-9 m high and 0.6 m in girth and is an ornamental plant. It is a native of Malaysia and is grown in many parts of India such as Punjab, Dehli, Bengal, Assam and the Andaman[1]. The bark and leaves are reported to cure diarrhoea, dysentery, snake bite, malaria, smallpox, eruptic fever, scabies, ulcer, and stomach disorders in children; in high doses it causes vomiting and mild diarrhoea[2]. The bark of *Sesbania grandiflora* possesses astringent, cooling bitter tonic, and anthelmintic and antipyretic properties. The fruits are believed to be laxative and stimulant. It has also been used in treatment of anaemia, bronchitis, fever, pain, thirst and tumours. The root is used for inflammation, the bark is astringent; leaves are alexeteric, anthelmintic and used for epilepsy, gout, itch and leprosy. The leaf is tonic and antipyretic and cures night blindness.

The objective of the present study was to investigate the antiulcer activity of ethanolic extracts leaves as it is traditionally reported to cure ulcer.

MATERIAL AND METHODS

Plant material

Leaves of *S. grandiflora were* collected from the western rural area of the Shirdi, Ahmednagar District (M. S.) during June at the flowering stage of the plant. It was authenticated by the Botanical Survey of India, Pune. (Voucher specimen no. CNASG1)

Preparation of extract

Shade-dried and powdered bark was defatted with petroleum ether and then extracted with ethanol in Soxhlet apparatus. Solvent evaporation under reduced pressure yielded the dried ethanolic extract (5.2%).

Animal used

Albino Wistar rats of either sex weighing between 150-250g were used. Animals were housed under standard conditions of temperature (24 ± 2°C) and relative humidity (30-70%) with a 12:12 light: dark cycle. The animals were given standard diet supplied by Pranav agro industries Ltd. (Sangli) and water ad libitum. All procedures involving animals were carried out under the institute ethics committee approval (997/c/06/CPCSEA).

Toxicity studies

Toxicity studies of the ethanolic leaf extract were carried out in Swiss Albino mice of either sex weighing between 20 and 25 g. The LD_{50} of the ethanolic leaf extract was found to be safe till 2000 mg/kg (i.p. and p.o.).

Antiulcer activity

Aspirin induced ulcer

Animals were divided in three groups of six animals each. Group I served as negative control received distilled water, Group II served as positive controls and received omeprazole at the dose of 20 mg/kg, and animals of Group III received ethanolic extract of leaves of S. grandiflora at the dose of 400 mg/kg, orally daily, respectively, for five days for ulcer protective studies. Aspirin in dose of 20 mg/kg was administration to the animals on the day of the experiment and ulcers were scored after 4 h. The animals were sacrificed and the stomach was then excised and cut along the greater curvature, washed carefully with 5.0 ml of 0.9 % NaCl and ulcers were scored by a person unaware of the experimental protocol in the glandular portion of the stomach. Ulcer index was then calculated by adding the total number of ulcers per stomach and the total severity of ulcers per stomach [3, 4, 5].

A score for the ulcer was made as: 0.5-Hemorrhage, 1-Streaks, 2-Spot ulcer, 3-Sever ulcer or Sever steaks, 4-Erosions, 5-Perforation.

Mean ulcer score for each animal was expressed as ulcer index. The percentage of ulcer protection was determined as follows:

$$\% \text{ Protective} = \frac{\text{Control mean ulcer index}}{\text{Control mean ulcer index} - \text{test mean ulcer index}} X100$$

Ethanol induced ulcer

The gastric ulcers were induced in rats of either sex weighing between 130-150 g by administrating absolute ethanol (8 ml/kg). They were kept in specially constructed cages to prevent coprophagia during and after the experiment. The rats were divided in to three groups each containing six animals and fasted for 24 h and allowed free access to water. The first group received control vehicle only and the second group received standard ranitidine in the dose of 20 mg/kg, third group received ethanolic extract of leaves of S. grandiflora (400 mg/kg body weight) orally daily, respectively, for five days for ulcer protective studies. On the sixth day of experiment the drugs were administered orally 30 min prior to the oral administration of absolute ethanol. The animals were anaesthetized 4 h latter with ether and stomach was incised along the greater curvature and ulceration was scored[6- 9]. The number of ulcers and the length of each ulcer were measured. A score for the ulcer was made as mentioned above.

Indomethacin induced ulcer

Animals are divided in three groups of six animals each. The gastric ulcers were induced by administering Indomethacin (5 mg/kg. p.o.) for five days. Group I served as negative control received distilled water, Group II served as positive controls received misoprostol (100 µg/kg, p.o), and animals of Group III received ethanolic extract of leaves of S. grandiflora at the dose of 400 mg/kg, orally daily, respectively, for another five days after the induction of ulcer. The rats were sacrificed on the fifth day of treatment and the ulcer index was determined. The glandular portion of the stomach was taken and used for estimation of ulcer. The number of ulcers and the length of each ulcer were measured. A score for the ulcer was made as mentioned above[6- 9].

Pyloric ligation method

In this method albino rats were fasted in individual cages for 24 h. Group I served as negative control received distilled water, Group II served as positive controls received ranitidine (20 µg/kg, p.o), and animals of Group III received ethanolic extract of leaves of *S. grandiflora* at the dose of 400 mg/kg, 1 h before pylorus ligation. Under light ether anesthesia, the abdomen was opened and the pylorus was ligated. The abdomen was then sutured. At the end of 4 h after ligation, the animals were sacrificed with excess of anesthetic ether, and the stomach was dissected out. Gastric juice was collected and its volume was measured. The glandular portion was then exposed and examined for ulceration. Ulcer index was determined. Total acid output was estimated from gastric juice collected from the 4 h pyloric ligated rats[10].

Estimation of total acid output

Total acid output of the gastric juice was estimated by titration of 0.1 ml of gastric juice with 0.01 N sodium hydroxide using phenolphthalein as indicator. Total acid output was expressed as mEq/L per 100 gm of body weight[10].

Statistical analysis

Mean values ± S. E. M. were calculated for each parameter. For the determination of significant intergroup differences, each parameter was analyzed separately and one-way analysis of variance (ANOVA) was carried out. $p<0.05$ was consider significant.

RESULTS AND DISCUSSION

Ulcer index parameter was used for the evaluation of anti-ulcer activity since ulcer formation is directly related to factors such as reduction in gastric volume, decrease in free and total acidity. Ethanolic extract of leaves of *S. grandiflora* at the dose of 400 mg/kg has decreased the intensity of gastric mucosal damage induced by ulcerogenic agents.

EELSG at the dose of 400 mg/kg and omeprazole at 20mg/kg produced a significant ($P < 0.05$) reduction in the ulcer index 21.41 and 18.5 and has protection index of 84.95 % and 87.00 % respectively as shown in table 1. Aspirin has been reported to produce ulcers by both local and systemic effects[11]. Aspirin causes direct irritant effect and mucosal damage by interfering with prostaglandin synthesis, increasing acid secretion by increasing the H^+ ion transport/back diffusion of H^+ ions, resulting overproduction of leukotrienes and other products of 5-lipoxygenase pathway[5]. It decreases mucin, surface active phospholipids bicarbonate secretion, mucosal proliferation and also produces damage by formation of free radicals[12]. The possible protective effect of EELSG against aspirin-induced gastric lesions could be due to prevention of direct irritation, increased mucus secretion and due to its 5-lipoxygenase inhibitory effect.

Table 1: Anti-ulcer activity of ethanolic extract of leaves of *S. grandiflora* on different ulcer-induced models

Treatment	Aspirin induced ulcer		Ethanol induced ulcer		Indomethacin induced ulcer		Pylorus ligated ulcer	
	Ulcer index	% protection	ulcer index	% protection	ulcer index	% protection	Ulcer index	% protection
Vehicle	142.33 ± 3.667	--	117.50 ± 4.463	--	87.83 ± 2.928	--	124.00 ± 2.733	--
EELSG 400mg/kg	21.41 ±1.819*	84.95	36.00 ± 2.745*	69. 36	25.75 ± 2.032*	70.68	48.25 ± 0.910*	61.08
Ranitidine 20mg/kg	§	§	32.0 ± 1.461*	72.26	§	§	32.0 ± 1.461*	74.19
Omeprazole 20mg/kg	18.5 ± 2.566*	87.00	§	§	§	§	§	§
Mesoprostol	§	§	§	§	16.833* ± 1.138	80.83	§	§

Values are mean ± S.E.M. *p<0.05, **P< 0.01, *** P< 0.0001compared to control group.

The group treated for the negative control and receive vehicle; § -not done. EELSG - ethanolic extract of leaves of *S. grandiflora*

Table2: Effect of ethanolic extract of leaves of *S. grandiflora* on Gastric volume, free acidity and total acidity of pylorus ligation induced-ulcer

Group	No. of Animals used	Gastric Juice ml	Total Acidity mEq/ltr
Control	6	2.7 ± 0.1000	122.40 ± 4.020
Ranitidine 20mg/kg	6	2.08 ± 0.0860*	63.2 ± 1.655*
EELSG 400 mg/kg	6	2.26 ± 0.0748***	72 ± 0.9274*

Values are mean ± S.E.M. *p<0.05 extremely significant, **P< 0.01 very significant, ***<0.0001 significant as compared to control group. EELSG - ethanolic extract of leaves of *S. grandiflora*

EELSG showed the ability to reduce significantly the severity of ulceration of stomach induced by absolute ethanol. EELSG at dose of 400 mg/kg and ranitidine at 20mg/kg has shown significant (P < 0.05) reduction in ulcer index upto 36.00 and 32.0 with protection index of 69. 36% and 72.26% respectively as shown in table 1. The results of histopathological investigation revealed that the pretreatment with EELSG absolutely prevented the ethanol-induced congestion, hemorrhage, edema, necrosis, inflammatory and dysplastic changes, erosions and ulceration in the gastric mucosa of rats. The stomach appearance was normal. The incidence of ethanol-induced ulcers predominant in the glandular part of stomach was reported to stimulate the formation of leukotriene C4 (LTC4), mast cell secretory products[13], and reactive oxygen species resulting in the damage of rat gastric mucosa[14]. In ethanol model, ulcers are caused due to perturbations of superficial epithelial cells, notably the mucosal mast cells leading to the release of the vasoactive mediators including histamine, thus causing damage to gastric mucosa[15]. Mucosal blood flow has been attributed to be an important factor in the damage caused by alcohol and is modulated by prostaglandin[16]. The effectiveness of EELSG protection against mucosal damage caused by ethanol is indication of its effect on prostaglandins. The results are presented in table 1.

EELSG at dose of 400 mg/kg has shown significant effect in indomethacin induced ulcer model with a protection index of 70.68% (P < 0.05) whereas standard drug misoprostol has 80.83 %. Indomethacin causes generation of reactive oxygen metabolites (such as superoxide anion, hydrogen peroxide and hydroxyl radical), which damages the gastric tissue and causes ulcer formation. The pathogenesis of gastric mucosal lesions by indomethacin is associated with increased lipid peroxidation. Oral administration of EELSG prevents gastric ulcers. Reduced glutathione in the gastric mucosa acts as the major scavenger of the oxygen-derived free radicals. It may be concluded that EELSG has preventive action on indomethacin induced ulcer in rats. It is possible that the antioxidant effect of EELSG might also play a role in the mechanism of antiulcer activity[17]. The results are summarized in table 1.

After 1h of treatment with EELSG, pylorus ligation of rats for 4h resulted in accumulation of gastric secretory volume, and increase in titrable acidity and ulceration (Table 2). EELSG has also showed significant effectiveness (P < 0.05) in pylorus ligation induced gastric ulcer model. It shows protection index of 61.08% at the dose of 400 mg/kg whereas standard drug omeprazole at 20mg/kg has shown 74.19 % protection. Total acidity of EELSG treated group was found to be 72 mEq/ltr, standard omepazole treated group 63.2 mEq/ltr which is less than that of negative control group which showed 122.40 mEq/ltr total acidity as shown in table 2. Pylorus ligation induced gastric ulcers occur because of an increase in acid-pepsin accumulation due to pyloric obstruction and subsequent mucosal digestion and breakdown of the gastric mucosal barrier[18]. A copious amount of mucus is secreted during superficial damage and provides favorable microenvironment in repair. Hence estimation of acid secretion, pepsin secretion and mucus secretion is a valuable part of the study to clarify the mechanism of action of the drug under trial.

The qualitative phytochemical study reveals the presence of alkaloids, carbohydrates, phytosterols, saponnins, tannins and flavonoids. The above effects of EELSG may also be due to the presence of tannins and flavonoids in the extract. Tannins have astringent action, precipitating proteins of mucosal membranes and skin. According to Tani *et al.* (1979) and Esaki *et al.* (1986) some tannins suppresses the gastric secretion, having a local action of protection of the gastric mucosa[19, 20].

Overall, EELSG has shown a substantial and significant protection against gastric ulcers in all the models. This protective effect might have been mediated by both anti-secretory and cytoprotective mechanisms. Moreover, further insight into the precise mechanism of action is essential to exploit the complete potency of EELSG and increase its usage in contemporary medicine.

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