Research Article

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ANTIULCER ACTIVITY OF *SPHAERANTHUS INDICUS* AGAINST INDOMETHACIN, ETHANOL AND SIX HOURS PYLORUS LIGATION INDUCED ULCER IN RATS

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ABSTRACT

The present study was conducted to test the antiulcer activity of ethanol extract of aerial parts of *Sphaeranthus indicus* (SIEE) in indomethacin, ethanol and 6 hours pylorus ligation induced ulcer models in rats. SIEE was prepared and subjected to acute toxicity study as per CPCSEA guideline number 420. Two doses i.e. 200 mg/kg and 400 mg/kg were selected. In pylorus ligation induced ulcer model, the parameters taken for assessing the anti-ulcer activity were gastric volume, pH, free acidity, total acidity and ulcer index. Ulcer index was also determined in ethanol and indomethacin induced ulcer models. Pretreatment with the extract has shown dose dependent decrease in ulcer index in all the experimental models of ulcers (indomethacin, ethanol and pylorus ligation induced ulcers) and also reduced the total acidity, free acidity, gastric volume and increased the pH in pylorus induced ulcer model. However, the results of gastric volume and pH were not significant with 200mg/kg dose. It is concluded from this study that SIEE possess antiulcer properties in different gastric ulcer models. The antiulcer properties of the extract may be attributed to the polyphenolic compounds that are present in plant.

Keywords: *Sphaeranthus indicus,* antiulcer, pylorus ligation, ethanol, indomethacin.

INTRODUCTION

Gastric ulcer is a common disorder of the gastrointestinal system, which causes much discomfort to patients, disrupting their daily routines and causing mental agony. Ulcers are caused due imbalances between the offensive and defensive factors of the gastric mucosa[1]. Although there are many products used for the treatment of gastric ulcers, most of these drugs produce several adverse reactions[2]. Hence herbal medicines derived from plant extracts are increasingly being utilized. Several natural drugs have been reported to possess anti-ulcerogenic activity by virtue of their predominant effect on mucosal defensive factors[3, 4]. Hence the present study was planned to exploit the safety and efficacy of a *Sphaeranthus indicus*.

Sphaeranthus indicus Linn belongs to family Asteraceae. The plant is reportedly used on treating epileptic convulsions, mental illnesses and hemicranias[5]. It is used as a tonic, laxative, digestive, anthelmintic, and the treatment of insanity, tuberculosis, diseases of the spleen, anemia, bronchitis, elephantiasis, pain of the uterus and vagina, piles, asthma, leucoderma and hemicranias[5,6]. Literature reports of this plant revealed the presence of an essential oil, glycosides, and eudesmanoids[7], an alkaloid sphaeranthine[8] and an isoflavone 5,4'-dimethoxy-3'-prenylbichanin 7-0-β-galactoside with some interesting sesquiterpine[9-11] and a new flavone glycoside[12].

The pharmacological data reveals that plant possess analgesic[13], anticonvulsant[14,15], hepatoprotective[16,17], anthelmintic[18], antimicrobial[19], antihyperlipidemic[20], anti-inflammatory[21,22], antioxidant[16,23], anti-diabetic[24] and neuroleptic activity[25].

Preliminary phytochemicals analysis of SIEE revealed the presence of flavonoids, tannins and saponins. There are reports that flavonoids and tannins have been found to be effective against ulcer in experimental animals[26]. Hence, the present study was undertaken with the aim to assess the antiulcerogenic properties of SIEE.

MATERIAL AND METHODS

Plant material and preparation of extracts

The aerial part of *Sphaeranthus indicus* were collected from fields of Tirupati, Andhra Pradesh. The plant was authenticated at Department of Botany, Sri venkateswara University, Tirupati, Andhra Pradesh and was given a voucher number DB/SVI/2011/989. The collected aerial part of *Sphaeranthus indicus* were air dried and powdered. The powdered of *Sphaeranthus indicus* was extracted with petroleum ether, chloroform and 70 % ethanol in a Soxhlet extractor. The residue was removed by filtration and the extract evaporated to dryness at a lower temperature under reduced pressure in a rotary evaporator. Preliminary phytochemical screening of ethanol extract of aerial part of *Sphaeranthus indicus* (SIEE) revealed the presence of saponins, glycosides, flavonoids, tannins, steroids and proteins.

Animals

Wistar albino rats (180-220g) and mice (18-25 g) of either sex were used for the study. Approval from the institutional animal Ethical committee

(1554/PO/a/11/CPCSEA) for usage of animal in the experiment was obtained as per the Indian CPCSEA guidelines.

Acute Toxicity studies

The acute toxicity was determined on albino mice by fixed dose method of OECD Guide line number 420 given by **CPCSEA**.

Anti- ulcer activity

Indomethacin induced ulcer

The albino rats of either sex weighing between 180 – 220 gm were divided into 4 groups of 6 animals each and fasted for 24 h with water *ad libitum* prior to experiment. The animals of group 1 were pretreated with vehicle and the animals of group 2 were treated with standard i.e. lansoprazole 8 mg/kg. Similarly the animals of group 3 and 4 were pre-treated with ethanol extract 200 mg/kg and 400mg/kg respectively. Indomethacin (30mg/kg p.o.) was administered to the animals of all groups, 60 minutes after the respective treatments. The animals were then sacrificed by cervical dislocation after 4 hrs. The stomach was taken out and cut open along the greater curvature[27]. The number of ulcers per stomach were noted and severity of the ulcers were observed microscopically and scoring was done[28]: 0 for normal coloured stomach, 0.5 for red coloration, 1 for spot ulcer, 1.5 for hemorrhagic streaks, 2 for ulcer between > 3 but < 5mm and 3 for ulcer > 5mm. Mean ulcer score for each animal was expressed as ulcer index. The percentage protection was calculated.

Ethanol (EtOH) induced ulcer

The albino rats of either sex weighing between 180 – 220 gm were divided into 4 groups of 6 animals each and fasted for 24 h with water *ad libitum* prior to experiment. The animals of group 1 were pretreated with vehicle and the animals of group 2 were treated with standard i.e. lansoprazole 8mg/kg. Similarly the animals of group 3 and 4 were pre-treated with ethanol extract 200 mg/kg and 400mg/kg respectively. Ethanol (100% 1ml/200 g, po) was administered to all the animals of all groups, 60 minutes after the respective treatments. The animals were sacrificed by cervical dislocation after one hour of EtOH administration and stomach was incised along the greater curvature and examined for ulcers[29,30]. The ulcer index was scored as mentioned above[27] and percentage protection was also reported.

Six hours Pylorus – ligated (PL) induced ulcers in rats

Albino rats of either sex weighing between 180 – 220 g were divided into 4 groups of 6 animals each and fasted for 18 h. Control vehicle (Group 1), standard drug (Group 2), extracts (Group 3 & 4) were administered 60 minutes prior to pyloric ligation under light ether anesthesia. The abdomen was opened and pyloric ligation was done without causing any damage to its blood supply. The stomach was replaced carefully and the abdomen wall was closed in two layers with interrupted sutures. The animals were deprived of water during the post operative period. After 6 hrs, stomach was dissected out; contents were collected into tubes for estimation of biochemical parameters. The stomach was taken out and cut open along the greater curvature and ulcers were scored and percentage protection was reported as mentioned in the above explained models[27, 31].

Gastric Secretion - The gastric juice was collected 6 hours after pylorus ligation and centrifuged for 5 minutes at 2000 rpm and the volume of supernatant was noted. The pH of the gastric juice was recorded by the pH meter. Free acidity and total acidity were determined using 0.01N NaOH and Topfer's reagent containing phenolphthalein as indicator[27].

Table 1: Effect of SIEE on Gastric Secretion following 6 hours Pyloric Ligation induced Ulcer in Rats

Treatment	Volume (ml)	pH	Free Acidity (Eq/l)	Total Acidity (Eq/l)
Control	4.48± 0.27	1.99 ± 0.23	30± 1.826	97.74± 9.178
Lansoprazole 8mg/kg Body weight.	1.77±0.22***	5.97± 0.41***	12.5±0.93***	27.33± 2.03***
SIEE 200mg/kg **Body weight**	3.63± 0.26 ns	4.60 ± 0.26***	25.16± 3.02ns	74±7.155*
SIEE 400mg/kg Body weight	3.25± 0.22*	5.62 ± 0.53***	21.33± 3.12ns	62 ± 3.58**

Values are the mean ± S.E.M. of six rats / treatment.
Significance nsP >0.05, *P <0.05, **P<0.001 and *** P<0.001 Vs. Control

Table 2: Effect of SIEE on Indomethacin, Ethanol (1ml/200 gm) and 6 hours Pylorus ligation (PL) induced gastric ulcers in rats

Treatment	Ulcer Index (% of protection)		
	Indomethacin	Ethanol	Pylorus Ligation
Positive Control	4.16 ± 0.48	6.25 ± 0.64	5.33 ± 0.9189
Lansoprazole 8mg/kg **Body weight**	1.33 ± 0.28*** (68.03)	1.42 ± 0.40*** (77.28)	0.83 ± 0.17*** (84.43)
SIEE 200mg/kg **Body weight**	2.5 ± 0.37ns (39.90)	2.58 ± 0.35*** (58.72)	2.33 ± 0.40*** (56.29)
SIEE 400mg/kg **Body weight**	1.67 ± 0.11*** (59.85)	2.17 ± 0.44*** (65.28)	1.92 ± 0.35*** (63.98)

Values are the mean ± S.E.M. of six rats /treatment.
Significance nsP >0.05 and *** P<0.001 Vs. Control

RESULTS

In the pylorus ligation induced ulcer model, SIEE significantly reduced the ulcer index, total acidity and raised the gastric pH in comparison to the control group. However no significant changes were observed with the free acidity and gastric volume (at 200 mg/kg dose) (Table 1). In the indomethacin induced ulcer model, the observations of positive control group indicated that indomethacin (30mg/kg) induced gastric ulcerations to the extent of 4.16 ± 0.48 (ulcer index). Pretreatment with test extracts reduced the ulceration in a dose dependant manner. The extent of gastro-protective effect of the test extracts was 39.90% and 59.85% at 200mg/kg and 400mg/kg doses respectively, which is comparable to that of standard lansoprazole 8mg/kg. Similar results were obtained with ethanol induced ulcer model also and the test extract has shown gastro-protection in a dose dependant manner i.e. 58.72% and 65.28 % protection at 200 and 400 mg/kg doses respectively. (8mg/kg) (Table 2).

DISCUSSION

In the pyloric ligation induced ulcer model, pyloric ligation -induced ulcers are thought to be due to increase in acid-pepsin accumulation due to pyloric obstruction and subsequent mucosal digestion[32]. The anti-ulcer property of SIEE in pylorus ligation model is evident from its significant reduction in free acidity, total acidity and ulcer index. Because SIEE treated animals significantly inhibited the formation of ulcers in the pylorus ligated rats and also decreased both the concentration and increased the pH, it is suggested that SIEE can suppress gastric damage induced by aggressive factors.

Several factors have been implicated in ethanol-induced gastric ulcers like products of arachidonate (LTC_4) metabolism, oxygen derived radicals, gastric mucosal blood flow, pepsinogen[33-34], whereas depression of gastric mucosal blood flow reduces bicarbonate secretion and mucus production, thus allowing back diffusion of hydrogen ions[35]. SIEE significantly protected the gastric mucosa against ethanol challenge as shown by reduced values of ulcer index as compared to control group suggesting its potent gastroprotective effect. Similarly NSAID'S like indomethacin inhibits COX_1 thereby inhibits the prostaglandin synthesis, consequently lipooxygenase pathway is enhanced liberating leukotrienes and these leukotrienes are reported to have a role in ulcerogenesis. In addition there is some evidence that NSAIDs may induce ulcer by causing the back diffusion of H^+ ion into mucosal cells[36,37]. Therefore the antiulcer effect of the SIEE may be due to its ability to inhibit the synthesis of prostaglandins/leukotrienes.

The phytochemical analysis of the SIEE revealed the presence of saponins and flavonoids, substances known to affect the integrity of mucous membranes. Flavonoids have also been reported to offer some protection in ulcer development by increasing capillary resistance and improving microcirculation[38].

In conclusion, our results showed that the antiulcer activity of the SIEE was perhaps a result of the interplay between its anti-secretory, cytoprotective and the antioxidant principle properties. Further investigation is going on to isolate, characterize and screen the active principles that possess anti-oxidant and gastroprotective properties.

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