Original Article

Evaluation of Gastric Anti-Ulcer Potency of Ethanolic Extract of *Sesbania Grandiflora* Linn Leaves in Experimental Animals

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ABSTRACT

Objective: To evaluate gastric anti-ulcer potency of ethanolic extract of *Sesbania grandiflora* Linn (EESG) (Fabaceae) leaves in experimental animals.
Methods: EESG was investigated in pylorous ligation and ethanol induced ulcer models in Wister rats. In both models ulcer index was determined as common parameters. The extract was ingested orally at doses of 250 and 500 mg/kg to the ulcer induced rats for determining the potency of ulcer inhibition.
Results: The extract (250 and 500 mg/kg) showed significant (P<0.05) reduction in gastric volume and ulcer index as compared to the control in both of the ulcer models.
Conclusions: It can be concluded that EESG possesses anti-ulcer effect as well as ulcer healing properties, which might be due to antisecretory properties.

Keywords: Antiulcer, Biochemical Parameter, *Sesbania grandiflora*, Pyloric ligation.

INTRODUCTION

Peptic ulcer is one of the most regular gastrointestinal disorder[1]. The formation of peptic ulcers depends on imbalance between acid and pepsin secretion and the mucosal defense factors[2]. Mucosal resistance can be disrupted due to injury caused by NSAIDs and *Helicobacter* pylori,[3] the most common causative pathogen for gastric ulcer in human. Increased gastric motility[4], vagal hyperactivity[5], mast cell degranulation[6], reduced flow of blood to the gastric mucosa[7] and decreased prostaglandin levels during conditions involving stress are involved in generation of gastric ulcers. Reactive oxygen species plays a role in experimental gastric damage induced by ischemia and reperfusion[8], hemorrhagic shock[9] and ethanol administration[10]. This has been rationale for the development of new

antiulcer drugs and search for novel molecule. Natural source of drugs is become the most interesting field for new investigation of herbal medicine for the treatment of various diseases including peptic ulcer. The objective of present study was to investigate the effectiveness of ethanolic extract of *Sesbania grandiflora* L. leaves in inhibiting the formation of gastric ulcer in experimental animals. The antiulcer drug is targeted at either counteracting aggressive factors like acid, pepsin, active oxidants, platelet aggravating factors (PAF), leukotrienes, endothelins, bile and exogenous factors including NSAIDs or stimulating the mucosal defenses like mucus, bicarbonate, normal blood flow, prostaglandins (PG) and nitric oxide[11]. The goals of treating peptic ulcer disease are to relieve pain, heal the ulcer and prevent ulcer recurrence. Currently there is no cost effective treatment that meets all these goals. Hence, efforts are on to find a suitable treatment from natural product sources.

Sesbania grandiflora (family: Fabaceae) is known as agati or the hummingbird tree (or scarlet wisteria), a small tree believed to have originated either in India or Southeast Asia and grows primarily in hot and humid tropical areas in the world. A native to Asian countries such as India, Malasia, Indonesia and the Philippines where it is commonly seen growing on the dikes between rice paddies, along roadsides and in backyards vegetable gardens[12]. The whole plant contains Grandifloral, arginine, cystine, histidine, isolucine, phenylalanine, tryptophan, valine, threonine, alanine, aspargine, aspartic acid and a saponin yielding oleanolic acid, galactose, rhamnose and glucuronic acid[13,14,15] and it also contains flavonol glycoside, kaempfrol[13,16]. The root-bark of the red-flowered variety is useful in vitiated condition of *vata* and arthralgia. The bark is astringent, cooling, bitter, tonic, anthelmintic and febrifuge. The pounded bark is externally applied to cure scabies. The juice of the bark is good for dyspepsia, diarrhea and gastralgia[17]. The leaves are acrid, bitter, sweet, cooling, aperient, tonic and diuretic and contain a non-poisonous saponine like substance. The leaf juice is used is nasal catarrh[18], nyctalopia and cephalagia. Leaves are chewed to disinfect mouth and throat and are useful in stomatalgia[14]. The flowers are cooling, bitter, astringent, acrid and antipyretic. The juice of the flowers is applied to the eyes for nyctalopia and is used for intermittent fevers. The fruits are sweet, bitter, laxative and alexiteric and are useful in flatulent-colic, astringent, cooling, bitter, tonic, anthelmintic, febrifuge, cure scabies, dyspepsia, diarrhea and gastralgia, astringent, antipyretic, for nyctalopia naemia, emaciation and vitated conditions of *tridosa*[12]. Ethanol extract of *Sesbania grandiflora* of both leaves and flowers showed anticancer activity in Swiss albino mice against Ehrlich Ascites Carcinoma cell line at the doses of 100 and 200 mg/kg i.p[19]. Antioxidant and Cardioprotective effect was evaluated in rats with the dose of 1000 mg/kg bw of *Sesbania grandiflora* aqueous suspension[20,21]. The leaf juice of *S. grandiflora* showed significant antiurolithiatic activity against calcium oxalate-type stones in rats[22]. The ethanol leaves extract showed significant protective effect against erythromycin estolate-induced hepatotoxicity[23]. The anticonvulsive activity of *S. grandiflora* leaves was evaluated using a variety of animal models of convulsions[24]. Wound healing activity of methanol extract of bark of *Sesbania grandiflora* (L.) Poir had been evaluated by using excision wound model in Wistar albino rats[25]. Seed oils of *Sesbania grandiflora* were investigated for their anthelmintic property against Pheritima pasthuma[26].

MATERIALS AND METHODS

Plant material

The leaves of *Sesbania grandiflora* were collected from Tripura, India in the month of October 2012. The plant was authenticated by Dr. A.P. Singh, Principal Investigator, Weed Control, Dept. of Agronomy, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India. A voucher specimen of the plant was preserved in the herbarium for further reference (Agro/WC/13/252).

Preparation of the extract

Leaves of *Sesbania grandiflora* Linn. were washed under running tap water and dried in shade for seven days. Dried leaves were mechanically reduced to a coarse powder and then sieved and stored in an air tight container at room temperature. The extraction method was based on the presence of active constituents in the drug, using various solvents ranging from non-polar to polar. Dried powder (500 g) was extracted sequentially with hexane, dichloromethane and 70% ethanol by using soxhlation method. The extracts were concentrated to dryness by distilling the solvent at low temperature using rotary evaporator. The extracts were preserved in refrigerator at 4^0C.

Acute toxicity study of the extract

Adult albino mice (25-30 g) were divided into four groups each containing three mice. The mice were fasted for 18 h with only access to water ad libitum before experiment. Group I, II and III were administered various doses of ethanolic extract of *Sesbania grandiflora* (EESG) i.e. 500, 1000, 5000 mg/kg. Group IV received distilled water only. All the doses and vehicle were administered by oral route. The animals were observed for 72 h for mortality[27].

Pyloric ligation induced gastric ulcer in rats

Animals were divided into four groups, each consisting of six rats. Rats in group I, served as control group, received distilled water (1 mL) orally. Rats in group II received omeprazole (20 mg/kg) which was used as a reference drug for ulcer protective studies. Rats in group III and IV were treated with EESG at doses of 250 and 500 mg/kg, respectively. After 45min of EESG and omeprazole treatment, pyloric ligation was done by ligating the pyloric end of stomach of rats of respective groups under ether anaesthesia at a dose of 35 mg/kg bw. Ligation was done without causing any damage to the blood supply of the stomach. Animals were allowed to recover and stabilize in individual cages and were deprived of water during postoperative period. After 4 h of surgery, rats were sacrificed and ulcer scoring was done. Gastric juice was collected and gastric secretion studies were performed[28,29].

Ethanol induced ulcer model

The ulcer was induced by administering ethanol. All the animals were fasted for 24 hours with free access of water before administration of ethanol. The animals were divided into four groups, each consisting of six rats. Rats in group I, served as control group, received distilled water (1 mL) orally. Rats in group II were administered with omeprazole (20 mg/kg) as a standard reference drug. Rats in group III and IV received EESG at doses of 250 and 500 mg/kg, respectively. The gastric ulcers were induced in rats by administrating absolute ethanol (90%) (1 mL/200 g) orally, after 45 min of EESG and omeprazole treatment. They were kept in specially constructed cages to prevent coprophagia during and after the experiment. The animals were anaesthetized 1 h later with anaesthetic ether and stomach was incised along the greater curvature and ulceration

will be scored. A score for the ulcer was study similar to pylorous ligation induced ulcer model[30].

Scoring of ulcer will be made as follows

Normal stomach.....(0)
Red coloration.....(0.5)
Spot ulcer.....(1)
Hemorrhagic streak..(1.5)
Ulcers.....(2)
Perforation.....(3)

Mean ulcer score for each animal will be expressed as ulcer index.

Percentage protection

$$\% \text{ Ulcer protection} = \frac{\text{Mean ulcer Index in Control} - \text{Mean ulcer index in Test}}{\text{Mean ulcer Index in Control}} \times 100$$

Calculation of ulcer index[31]

$U1 = (UN + US + UP) \times 10^{-1}$
U1 = Ulcer index
UN = Average of number of ulcer per animal
US = Average of severity score
UP = Percentage of animal with ulcer

Statistical analysis

The Dunnett's test was employed for statistical comparison. In all the cases, values of $P < 0.05$ were considered significant. All values were presented as mean ± SEM.

RESULTS

Pyloric ligation induced gastric ulcer in rats

In pyloric ligation induced ulcer model, oral administration of EESG in two different doses showed significant reduction in ulcer index, gastric volume, as compared to the control group. It was showing protection index of 47.86% and 77.78% at the doses of 250 and 500 mg/kg, respectively in comparison to control whereas omeprazole as reference standard drug showed reduction of ulcer 88.89% (Table 1 and Figure 1).

Ethanol induced gastric ulcer in rats

In control animal, oral administration of absolute ethanol produced characteristic lesions in the glandular portion of rat stomach which appeared as elongated bands of thick, black and dark red lesions. EESG showed significant protection index of 50% and 74.22% with the doses of 250 and 500 mg/kg, respectively in comparison to control. Omeprazole as reference standard drug showed reduction of ulcer 87.11% (Table 2 and Figure 2).

DISCUSSION

The etiology of peptic ulcer is unknown in most of the cases, yet it is generally accepted that it results from an imbalance between aggressive factors and the maintenance of mucosal integrity through the endogenous defence mechanism[32].

Pylorus ligation induced ulcer was used to study the effect of leaves extracts on gastric acid secretion and mucus secretion. The ligation of the pyloric end of the stomach causes accumulation of gastric acid in the stomach. This increase in the gastric acid secretion causes ulcers in the stomach. The original Shay rat model involves fasting of rats for 36 h followed by ligation of pyloric end of the stomach. The ulcer index is determined 5 h after pylorus ligation. The lesions produced by this method are located in the lumen region of the stomach. Many authors have modified the original model. In the present study, the Shay rat model described by Kulkarni was followed. Ethanol induced gastric ulcer was employed to study the cytoprotective effect of the extracts. Ethanol induced gastric lesion formation may be due to stasis in gastric blood flow which contributes to the development of the haemorrhage and

necrotic aspects of tissue injury. Alcohol rapidly penetrates the gastric mucosa apparently causing cell and plasma membrane damage leading to increased intracellular membrane permeability to sodium and water. The massive intracellular accumulation of calcium represents a major step in the pathogenesis of gastric mucosal injury. This leads to cell death and exfoliation in the surface epithelium[33, 34].

In conclusion, ethanolic extract of *Sesbania grandiflora* Linn (EESG) possesses anti-ulcer activity which might be due to its antisecertory and cytoprotective nature.

Conflict of interest

There is no conflict of interest.

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Table 1: Effect of *Sesbania grandiflora* Linn leaves extract on various parameters in pyloric ligation induced gastric ulcer in rats.

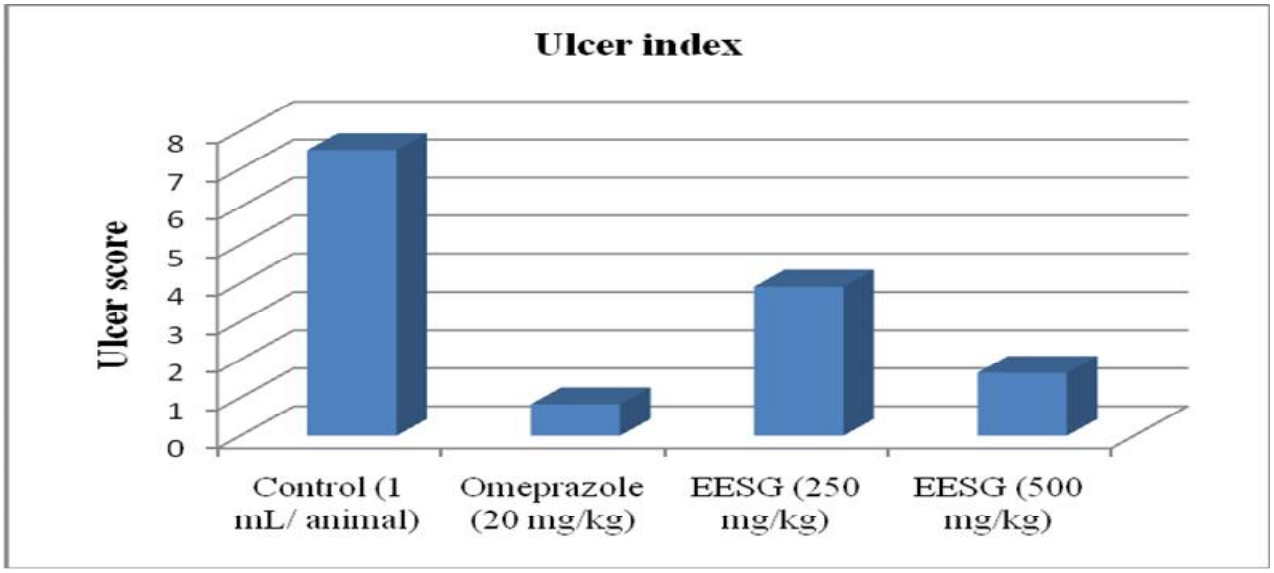
Groups	Treatment	Doses (mg/kg)	Ulcer index	% Protection	Volume of gastric juice (mL)	pH of gastric juice
I	Control	1 mL/ animal	7.5 ± 0.51	-	4.86 ± 0.11	1.7 ± 0.09
II	Omeprazole	20	0.83 ± 0.21*	88.89	2.18 ± 0.07*	5.3 ± 0.14*
III	EESG	250	3.91 ± 0.35	47.86	4.18 ± 0.07	2.71± 0.10*
IV	EESG	500	1.66 ± 0.33*	77.78	3.08 ± 0.04*	4.05 ± 0.08*

*: P<0.05 as compared to control group.

Table 2: Effect of *Sesbania grandiflora* Linn leaves extract on various parameters in ethanol induced gastric ulcer in rats.

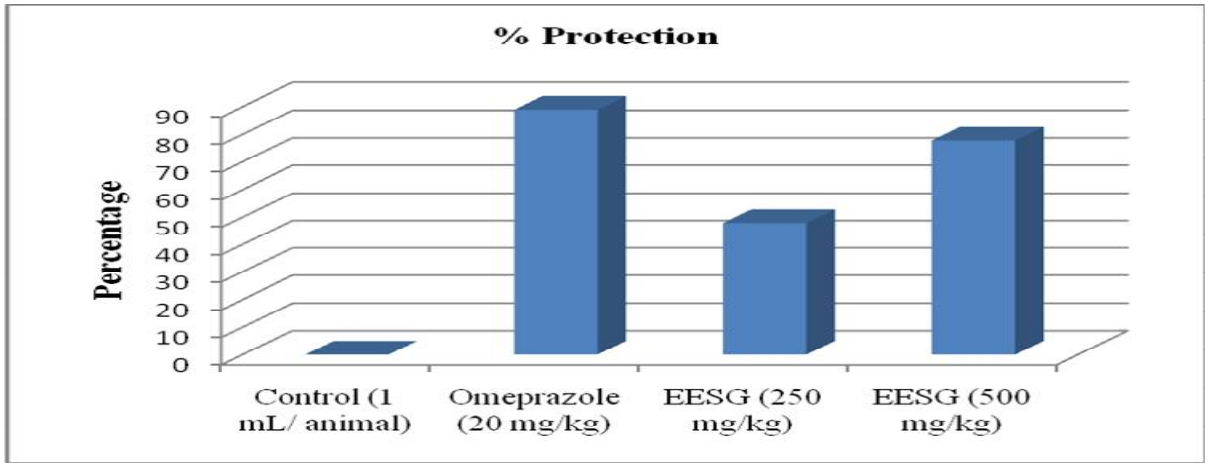
Groups	Treatment	Doses (mg/kg)	Ulcer index	% Protection	pH of gastric juice
I	Control	1 mL/	4.5 ± 0.34	-	2.1 ± 0.20
II	Omeprazole	20	0.58 ± 0.08*	87.11	4.6 ± 0.15*
III	EESG	250	2.25 ± 0.33	50.0	3.1 ± 0.12*
IV	EESG	500	1.16 ± 0.21*	74.22	4.2 ± 0.18*

*: P<0.05 as compared to control group.

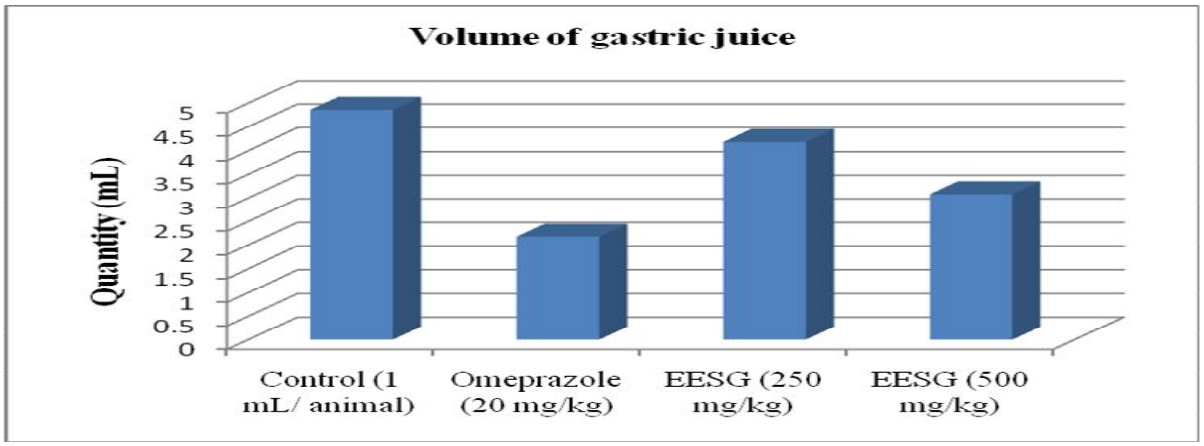


(A) Ulcer index in pyloric ligation induced gastric ulcer in rats.

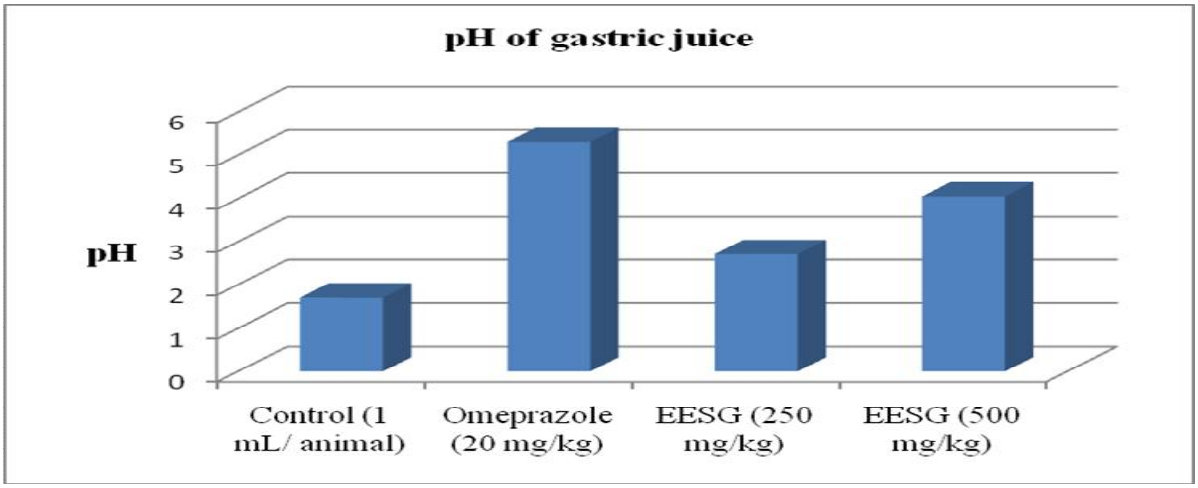
Fig 1: Effect of *Sesbania grandiflora* Linn leaves extract on various parameters in pyloric ligation induced gastric ulcer in rats.



(A) Percentage of protection in pyloric ligation induced gastric ulcer in rats.

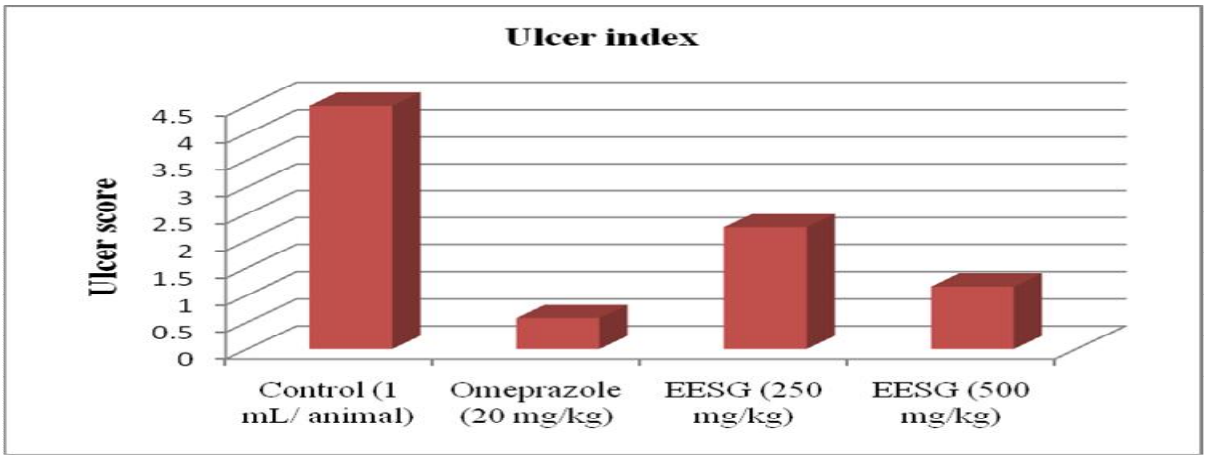


(C) Effect on gastric juice in pyloric ligation induced gastric ulcer.

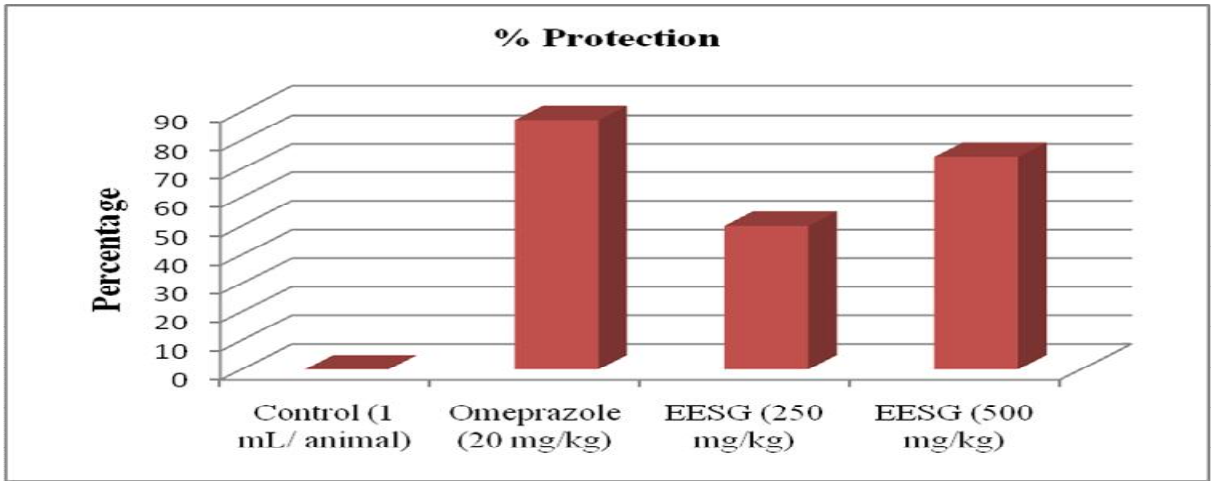


(D) Effect on pH of gastric juice in pyloric ligation induced gastric ulcer.

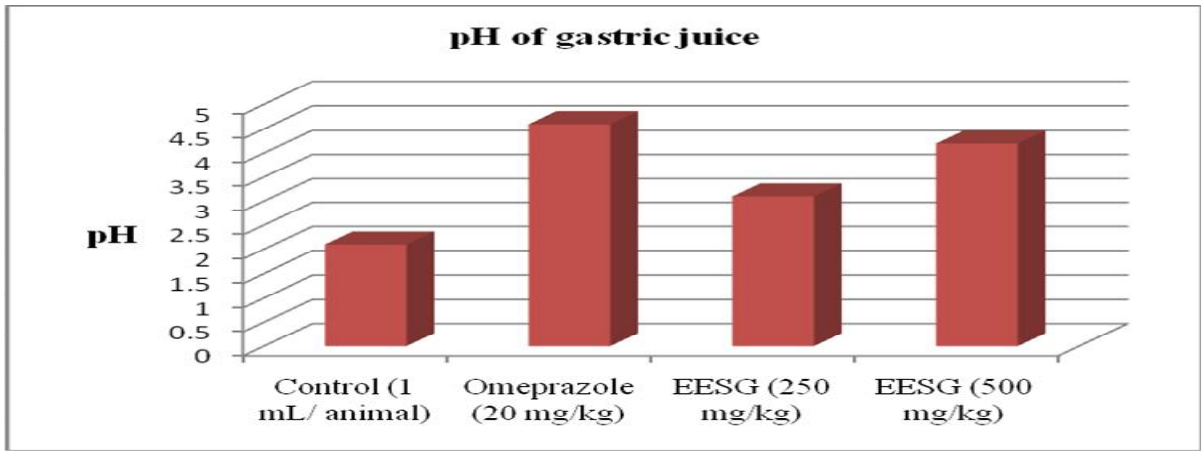
Fig 1: Effect of *Sesbania grandiflora* Linn leaves extract on various parameters in pyloric ligation induced gastric ulcer in rats.



(B) Ulcer index in ethanol induced gastric ulcer in rats.



(B) Percentage of protection in ethanol induced gastric ulcer in rats.



(C) Effect on pH of gastric juice in ethanol induced gastric ulcer.

Fig 2: Effect of *Sesbania grandiflora* Linn leaves extract on various parameters in ethanol induced gastric ulcer in rats.