ANTIULCER ACTIVITY OF THE METHANOLIC EXTRACT OF *SESBANIA GRAINDIFLORA*

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ABSTRACT

The anti ulcerogenic activity of *Sesbania graindiflora* was evaluated by employing aspirin and pylorus ligation induced ulcerations in rats. Non-steroidal anti-inflammatory drugs (NSAIDs) like aspirin are known to induce gastric ulceration. The reason being attributed principally to inhibition of biosynthesis of 'cytoprotective prostaglandins' (by inhibition of cyclo-oxygenase pathway of arachidonic acid metabolism), resulting in overproduction of leukotrienes and other products of 5-lipoxygenase pathway .Hence, the protective action of Sesbania graindiflora against aspirin- induced gastric lesions could possibly be due to its 5-lipoxygenase inhibitory effect.

KEY WORDS: - *Sesbania graindiflora,* pyloric ligation, aspirin induced ulcer.

INTRODUCTION

Sesbania graindiflora Linn (Fabaceae), popularly known as 'Basna' is an ornamental plant and is found in the plains of western Himalaya to Sri Lanka [1, 2].The various part plant is reported to cure diarrhoea, dysentery, paludism, snake bite, malaria, smallpox, eruptic fever, scabies, ulcer, and stomach disorders in children; in high doses it causes vomiting and mild diarrhea [3].Gastric ulcers arise due to various factors [4]. Even though the etiology of gastric ulcers is still debated, it is accepted that ulcers are caused due to net imbalances in mucosal offensive and defensive factors [5]. Ulcer therapy is now mainly focused on limiting the deleterious effects of offensive acid secretion, but search for new safer alternative drugs have rekindled the interest in cytoprotective drugs, which protect the gastric mucosa from damaging agents without influencing acid secretion or neutralizing intragastric acidity [6]. Although few drugs like sucralfate and prostaglandin analogs, i.e.misoprostol are recognized as cytoprotective agents [7], many natural drugs have been reported to possess this activity [8, 9]. Change in gastric emptying has been reported to be responsible for genesis of ulcers [10]. Free radical shave been reported to be responsible for many ailments including gastro duodenal ulcers [11].

The objective of the present study was to investigate the Antiulcer activity of its flower methanolic extract of different strength when administered by the oral route in rats.

MATERIAL AND METHODS

Plant material

Flowers of S. graindiflora were collected in the month of Oct-Nov ,2009 were used for the study. The plant was authenticated by Dr.M.Islam, Professor of Life Science, Dibrugarh University , Assam, India. The flowers were air dried at room temperature. The dried flowers were grounded to coarse powder and stored in air tight container.

Preparation of extract

The coarse powder was packed into soxhlet column and extracted successively with petroleum ether (60-80°C) and 70% methanol (60°C). The extracts were concentrate by using rotary flash evaporator under reduced pressure. The dried extracts were stored in air tight container in refrigerator below 10°C.

Experimental Animals

Albino rats (Wistar strain) of either sex weighing 200-250g and albino mice weighing 20-25g of either sex were used for the study. They were procured from DRDU, Gwalior, M.P. They were kept in the departmental animal house at 26 ± 2°C and relative humidity 55 ± 15%, light and dark cycles of 12 and 12 h, respectively for 10 days before and during the experiments. Animals were provided with standard rodent pellet diet (Golden feeds, Delhi) and water *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC) constituted under CPCESA (1252/ac/09/CPASEA).

Acute toxicity studies

The acute toxicity for 70% methanolic extract of flowers was determined in albino mice, maintained under standard conditions. The animals were fasted overnight, prior to the experiment, fixed dose method was adopted as per OECD Guideline No.420; (Annexure-2d) of CPCSEA.[12], The animals were observed for toxic symptoms such as behavioral changes, locomotion, convulsions and mortality for 72 h [13].

Phytochemical screening

Phytochemical screening was carried out according to Matos [14].

Pylorus ligation method

The rats were given the plant extract (1 g/kg) or cimetidine (11.5 mg/kg) 1 h before pylorus ligation. Six hrs after the ligation, the animals were killed and the stomach removed. The gastric contents were collected, centrifuged and the supernatant measured. The ulcer formed in the gastric mucosa were measured and scored [15]. The ulcer index, the percentage ulcerated surface and the percentage of inhibition were estimated as described above.

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. The percentage of ulcer protection was determined as follows

$$\% \text{ protective} = \frac{\text{Control mean ulcer index} - \text{test mean ulcer index}}{\text{Control mean ulcer index}} \times 100$$

Determination of Acidity

$$\text{Acidity} = \frac{\text{Vol. of NaOH} \times \text{Normality of NaOH} \times 100}{0.1} \text{ (mEq/L)}$$

Aspirin induced gastric ulcer

Aspirin (0.2 g/kg x 3 days) were administered once per day to groups of animals for the number of days specified [16]. Overnight fasted animals were sacrificed by cervical dislocation one hour after the last dose of ulcerogen. The stomach was incised along the greater curvature and examined for ulcers.

Statistical analysis

The experimental data were tested for statistical significance by means of analysis of variance with one way classification. Sequential differences among means were calculated at a level of $p<0.05$ using Tukey's contrast analysis. The ED50 was determined according to linear regression model [17].

RESULTS

Phytochemical screening

The result of preliminary phytochemical screening of methanolic extract of *Sesbania graindiflora* revealed that presence of saponins, tannins and triterpenes.

Pyloric ligation induced gastric ulcer

In pyloric ligation induced ulcer model, Oral administration of extract in two different dose showed significant reduction in ulcer index, gastric volume, free acidity, total acidity as compared to the control group. It was showing protection index of 75% and 89% at the dose of 250mg/kg and 500mg/kg respectively in comparison to control whereas Omeprazole as reference standard drug was reduction of ulcer 84%. (Results are tabulated in Table-1).

Aspirin induced gastric ulcer

In aspirin induced ulcer model, Oral administration of extract in two different dose showed significant reduction in ulcer index, gastric volume, free acidity, total acidity as compared to the control group. It was showing protection index of 50% and 64.34% at the dose of 250mg/kg and 500mg/kg respectively in comparison to control whereas Omeprazole as reference standard drug was reduction of ulcer 70.42%. (Results are tabulated in Table-2).

DISCUSSION AND CONCLUSION

In most of the cases the etiology of the ulcer is unknown. It is generally accepted that it results from an imbalance between aggressive factors and the maintenance of the mucosal integrity through the endogenous defense mechanism [18]. To regain the balance, different therapeutic agents including plant extracts are used to inhibit the gastric acid secretion or to encourage the mucosal defense mechanisms by increasing mucus production, stabilizing the surface epithelial cells, or interfering with the prostaglandin synthesis.

Even though many products in the market for the treatment of gastric ulcers, including antacids, proton pump inhibitors, anticholinergics and histamine H2-antagonists, are used, most of these drugs produce several adverse reactions, such as gynecomastia, hematopoietic changes, acute interstitial nephritis [19], thrombocytopenia [20], anaphylaxis reactions [21], nephrotoxicity and hepatotoxicity [22]. Medicinal plants are amongst the most attractive sources of new drugs, and have been shown to give promising results in treatment of gastric and duodenal ulcers.

The anti ulcerogenic activity of *Sesbania graindiflora* was evaluated by employing aspirin and pylorus ligation induced

ulcerations in rats. Non-steroidal anti-inflammatory drugs (NSAIDs) like aspirin are known to induce gastric ulceration. The reason being attributed principally to inhibition of biosynthesis of 'cytoprotective prostaglandins' (by inhibition of cyclo-oxygenase pathway of arachidonic acid metabolism), resulting in overproduction of leukotrienes and other products of 5-lipoxygenase pathway [23].Hence, the protective action of Sesbania graindiflora against aspirin-induced gastric lesions could possibly be due to its 5-lipoxygenase inhibitory effect. The plant can be further screened against various diseases in order to find out its unexplored efficacy and can be a potential source of biologically important drug candidates. Further studies on possible mechanism of different disease and isolation of active principle(s) responsible for different pharmacological activities are currently under progress in our laboratory.

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Table 1: Effect of *Sesbania graindiflora* flower extract on various parameters in pyloric ligation induced gastric ulcer.

Group	Treatment	Ulcer index (A)	Protection (%)	Gastric juice (mL) (B)	Free acidity (mEq/lit) (C)	Total acidity (mEq/lit) (D)
I	Control (Pyloric ligation)	14.5±1.3	-------	10.2±1.5	99.2±1.1	124±0.3
II	Cimetidine (11.5 mg/kg)	2.4±.05	84	2.4±.18	32.8± 2.4	57.8±1.4
III	Extract (250mg/kg)	3.6±1.2	75	4.2±.25	49.8±1.3	69.8±.35
IV	Extract (500mg/kg)	2.5±.09	89	3.9±0.56	42.6±1.9	63.7±1.6

n = 6 animals in each group; Values are mean±SEM.

(A) Homogeneous Populations, groups ranked

Gp 1 refers to GROUP=Grp 1
Gp 2 refers to GROUP=Grp 2
Gp 3 refers to GROUP=Grp 3

Gp 4 refers to GROUP=Grp 4

Gp Gp Gp Gp
2 4 3 1

This is a graphical representation of the Tukey multiple comparisons test. At the 0.05 significance level, the means of any two group underscored by the same line are not significantly different.

(B) Homogeneous Populations, groups ranked

Gp 1 refers to GROUP=Grp 1

Gp 2 refers to GROUP=Grp 2

Gp 3 refers to GROUP=Grp 3

Gp 4 refers to GROUP=Grp 4

Gp Gp Gp Gp
2 4 3 1

This is a graphical representation of the Tukey multiple comparisons test. At the 0.05 significance level, the means of any two groups underscored by the same line are not significantly different.

(C) Homogeneous Populations, groups ranked

Gp 1 refers to GROUP=Grp 1

Gp 2 refers to GROUP=Grp 2

Gp 3 refers to GROUP=Grp 3

Gp 4 refers to GROUP=Grp 4

Gp Gp Gp Gp
2 4 3 1

This is a graphical representation of the Tukey multiple comparisons test. At the 0.05 significance level, the means of any two groups underscored by the same line are not significantly different.

(D) Homogeneous Populations, groups ranked

Gp 1 refers to GROUP=Grp 1

Gp 2 refers to GROUP=Grp 2

Gp 3 refers to GROUP=Grp 3

Gp 4 refers to GROUP=Grp 4

Gp Gp Gp Gp
2 4 3 1

This is a graphical representation of the Tukey multiple comparisons test. At the 0.05 significance level, the means of any two groups underscored by the

same line are not significantly different.

1. **Table 2: Effect of alcoholic extract of *Sesbania graindiflora* Linn. on aspirin-induced gastric ulcer in rats.**

Group	Treatment	Dose	Ulcer score	Percentage protection From ulcer
I	Control	2ml/kg	3.3±0.92	--------
II	Cimetidine	11.5mg/kg	1.2±0.01	70.42
III	Extract	250mg/kg	2.1±0.042	50.00
IV	Extract	500mg/kg	1.3±0.31	64.34

n = 6 animals in each group; Values are mean±SEM.

Homogeneous Populations, groups ranked

Gp 1 refers to GROUP=Grp 1

Gp 2 refers to GROUP=Grp 2

Gp 3 refers to GROUP=Grp 3

Gp 4 refers to GROUP=Grp 4

Gp 2	Gp 4	Gp 3	Gp 1
———			
	———		
		———	

This is a graphical representation of the Tukey multiple comparisons test. At the 0.05 significance level, the means of any two group underscored by the same line are not significantly different.