

EVALUATION OF LEAF EXTRACT OF *SPATHODEA CAMPANULATA*.P.BEAUVE FOR GASTROPROTECTIVE ACTIVITY BY ETHANOL INDUCED ULCER MODELSoumya Nijaguna Hiremath^{1*}, Jagadeesha A. S.¹, A. Veeranagouda¹ and Ittagi Shanmukha¹

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ABSTRACT

Peptic ulcer is one of the major disease affecting the human population. It develops due to the imbalance between aggressive factors like acid, pepsin, *H. pylori* and bile salts and defensive factors like mucous, bicarbonate, blood flow, epithelial cell restoration and prostaglandins. Ulcers are more common in adult males and it occurs commonly at old age and in lower socio-economic class of individuals. It almost affects 9.5% women population and 10.5% men. This study is designed to determine the gastro protective activity of leaf extract of *Spathodea Campanulata* P. Beauv in ethanol induced ulcer model. The various extracts were prepared by using petroleum ether, chloroform, 70% ethanol and aqueous as a solvents. The 70% ethanolic extract (EELSC) was used to screen the gastro protective activity because of presence of higher concentration of flavonoids and polyphenols. The 70% EELSC was screened for anti ulcer activity induced by ethanol. The various relevant parameters like ulcer index, gastric volume, gastric PH, free acidity and total acidity were estimated in ulcer model. *In-vivo* antioxidants i.e. GSH, LPO, SOD and CAT were also carried out in stomach tissue. Pretreatment with 70% EELSC exhibited significant ulcer protective property at all the tested doses of 250, 333 and 500 mg/kg in ethanol induced gastric ulcer model. Anti-secretory studies in rats revealed that, the dose 333mg/kg significantly reduced total acidity, free acidity, gastric output and increases the pH of gastric secretion as compared to the other two doses. The test extract significantly increased levels of GSH, SOD and CAT and reversed the LPO level. The gastroprotective property may be attributed to the polyphenolic compounds like flavonoids and tannins that are present in the leaves of *Spathodea campanulata* P. Beauv.

KEYWORDS: Gastroprotective; Antioxidant; ethanol; Leaves.**INTRODUCTION**

In this modern world, gastrointestinal disorders are the universal problem. Peptic ulcer is also known as acid peptic disease (APD), an ulceration of the mucous membrane of the stomach and duodenum. An ulcer is a sore or erosion that forms when the lining of the digestive system is corroded by acidic digestive juices and thus extremely painful.^[1] Although the exact cause of ulceration is not known, hydrochloric acid and pepsin are responsible for maintaining the lesion once it is produced. Peptic ulceration occurs only in areas which are bathed by the acidic gastric juice. Therefore, the term peptic ulcer refers to ulceration of the areas which might be acted upon by acid peptic juice namely the stomach and the first portion of duodenum.^[2] Gastric ulcer affects a consider number of people worldwide and in the united states, approximately 50,000 persons are affected by gastric ulcer each year.^[2] Duodenal ulcer is most frequent in the individuals of age group 30 to 55 years.

Nowadays people are subjected to increase in stress due to the modern life style and they often consume fast

foods. These factors lead to many kinds of gastro intestinal disorders. About 10% of the population may develop peptic ulcer in their life time.^[3] Peptic ulcer disease encompassing gastric ulcer and are induced by several factors, including stress, smoking, nutritional deficiencies and ingestion of nonsteroidal anti-inflammatory drugs.^[4] The free radicals seems to play an important role in ulcerative and erosive lesions of the gastrointestinal tract,^[5] as they attack and damage many biological molecules. Therefore, treatment with antioxidants and free radical scavengers can decrease ethanol induced gastric mucosal damage.^[6-7] The plant products are becoming more popular than the synthetic drugs due to its low toxicity and effectiveness.^[8] Flavonoids and phenolic compounds are widely distributed in plants which have been reported to exert multiple biological effects, including antioxidant, free radical scavenging abilities, anti-inflammatory, anticarcinogenic etc.,^[9] In this view, we have selected one of such herb called as *Spathodea campanulata*. This plant have Several medicinal properties which includes flavonoids and reported many uses in folk medicine. The

literature survey revealed that, so far no scientific studies were carried out on gastro protective activity on leaves of *Spathodea campanulata* P. Beauv. Hence, in the present study, we were focused to evaluate the gastro protective activity of selected plant.

MATERIALS AND METHODS

Plant material and extraction: *Spathodea campanulata* P. Beauv leaves was collected from local garden. The plant was identified and authenticated by Prof. K. Prabhu, Department of Pharmacognosy, S.C.S. College of Pharmacy, Harapanahalli. A voucher Specimen has been deposited at the museum of the college. The Leaves were dried in shade at room temperature. The dried Leaves were coarse powdered and packed in soxhlet column and extracted successively with petroleum ether (60-80° C), 70% ethanol (70° C) and distilled water at room temperature. The extracts were concentrated under reduced pressure (bath temperature 50°C) and stored in airtight container. The ethanolic extract was selected for the present study.

Experimental Animals: Albino rats (180-250g) of either sex were used throughout the experiments and they were procured from Venkateshwara enterprises Bangalore. Before initiation of experiment, the rats were acclimatized for a period of 7 days. Standard environmental conditions such as temperature at (27° ± 2° C), relative humidity (45-55%) and 12 h dark/light cycle were maintained in the quarantine. All the animals were fed with rodent pellet diet (Gold mohr, Lipton India Ltd.) and water was allowed *ad-libitum* under strict hygienic conditions. Ethical clearance for performing the experiments on animals was obtained from Institutional Animal Ethics Committee (IAEC) (Ref. no. SCSCP /619/4/2013-14) prior to the beginning of the activity.

Fixation of dose:^[10] Based upon previous literature of the leaves, the plant doses were selected for the pharmacological screening, Which are as follows 250mg/kg, 333mg/kg, 500mg/kg.

Experimental Design:^[11] Albino rats of either sex weighing between 200 – 250 g were selected and divided into 5 groups of 6 animals each.

- Group I - Positive control (Ethanol 5.0ml/kg p.o)
- Group II - Standard (Pantoprazole 20 mg/kg i.p.)
- Group III - 70% EELSC 250mg/kg p.o
- Group IV - 70% EELSC 333mg/kg p.o
- Group V - 70% EELSC 500mg/kg p.o

The animals were fasted for 24 hrs with free access to water. Animals were given different doses of 70% EELSC and standard drug Pantoprazole as mentioned above. Thirty minutes after the treatment the animals were treated with 5ml/kg absolute alcohol to induce ulcers. Animals were sacrificed 6 hr after ethanol administration under mild ether anaesthesia. Stomachs were isolated and cut open along the greater curvature and pinned on a soft board. The numbers of ulcer per stomach were noted & severity of the ulcer scored microscopically with the help of hand lens (10x). The ulcer index was measured. The isolated stomach was used for the estimation of tissue Glutathione levels (GSH)^[12] lipid peroxidation (LPO),^[13] catalase (CAT),^[14] and superoxide dismutase (SOD)^[15]

Statistical analysis Results were expressed as mean ± SEM, (n=6). Statistical analysis were performed with one way analysis of variance (ANOVA) followed by Tukey's Kramer comparison test by using Graph Pad Instat Software, version 5.0. P value less than 0.05 was considered as statistically significant.

RESULTS

70% EELSC showed significant antiulcer activity in a median dose when compared to positive control, which is evident by decrease in ulcer index of 70% EELSC at a dose of 250 mg/kg, is 0.700±0.211 and at is 333mg/kg is 0.533±0.0989 and at 500mg/kg is 0.750±0.0922, whereas standard ulcer index is 0.483±0.105. The results are summarized in table1.

Table 1: Effect of 70% EELSC on ethanol induced ulceration in rats.

Groups	Treatment	Dose	Mean ulcer index ± SEM	% Protection	Mean vol. of Gastric Juice(ml) ±SEM	Mean Gastric pH ±SEM
I	Positive control	5ml/kg	1.40±0.100	--	6.82±0.192	4.10±0.325
II	Standard(Pantoprazole)	20mg/kg	0.483±0.105***	65%	3.67±0.313***	6.92±0.226***
III	70% EELSC	250mg/kg	0.700±0.211**	50%	5.10±0.324**	5.92±0.381**
IV	70% EELSC	333mg/kg	0.533±0.0989***	61%	4.83±0.435***	6.35±0.246***
V	70% EELSC	500mg/kg	0.750±0.0922**	46%	5.52±0.276*	5.55±0.313**

The data are presented as mean± SEM, n=5. Levels of significance: *P< 0.05, **P<0.01, ***P<0.001 compared to control group.

The effect of *In vivo* antioxidant status revealed marked depletion of GSH, SOD, CAT levels in positive control group, when it is pretreated with 70% EELSC it showed increase in the levels of tissue GSH, SOD

and CAT. There was also inhibition of lipid peroxidation levels in the pretreated groups. The results are depicted in table.2 and 3.

Table 2: Effect of 70% EELSC on GSH, LPO level in ethanol induced ulceration in rats.

Treatment	GSH		LPO	
	Mean \pm SEM	% increase	Mean \pm SEM	% inhibition
Positive control	0.0648 \pm 0.00586	-----	0.45 \pm 0.029	-----
Standard(Pantoprazole)	0.0988 \pm 0.00411***	52.00%	0.28 \pm 0.022	37.70%
70% EELSC(250 mg/kg)	0.0882 \pm 0.00678*	36.00%	0.34 \pm 0.015	24.40%
70% EELSC(333 mg/kg)	0.0914 \pm 0.00445**	41.00%	0.33 \pm 0.031	26.00%
70% EELSC(500 mg/kg)	0.0921 \pm 0.00657**	42.00%	0.31 \pm 0.015	31.10%

The data are presented as mean \pm SEM, n=6 Levels of significance: *P< 0.05, **P<0.01, ***P<0.001 compared to control group.

Table 3: Effect of 70% EELSC on SOD and CAT level in in ethanol induced ulceration in rats.

Treatment	SOD		CAT	
	Mean \pm SEM	% increase	Mean \pm SEM	% increase
Positive control	2.98 \pm 0.375	-----	3.07 \pm 0.349	-----
Standard (Pantoprazole)	6.26 \pm 0.482***	96.34%	6.26 \pm 0.482***	89%
70% EELSC (250 mg/kg)	4.94 \pm 0.357*	65%	4.94 \pm 0.357*	60%
70% EELSC (333 mg/kg)	5.63 \pm 0.576**	88%	5.63 \pm 0.576**	83%
70% EELSC (500 mg/kg)	5.78 \pm 0.679**	90%	5.78 \pm 0.679**	88%

The data are presented as mean \pm SEM, n=6 Levels of significance: *P< 0.05, **P<0.01, ***P<0.001 compared to control group.

DISCUSSION

Human beings constantly struggle against the changing environment condition to maintain optimum health. It is increasing being realized now that a majority of the disease/disorders are mainly due to imbalance between pro-oxidant (free radicals) and anti-oxidant homeostatic phenomenon.^[16] Several antioxidants of the plant origin are experimentally proved and used as effective protective agents against oxidative stress.^[17] The leaves were collected and extracts were prepared. Thus prepared extracts were subjected to preliminary phytochemical investigation; the results exhibited that the leaves possess carbohydrates, flavonoids, tannins, and saponins in 70% ethanolic extract. In the ethanol induced ulcer model, Ethanol provoked gastric mucosal lesions are caused by the direct toxic effects of ethanol through the reduction in mucus production, gastric mucosal blood flow and bicarbonate secretion. Endogenous glutathione and prostaglandin levels are also lowered by ethanol while the release of histamine, influx of calcium ions, generation of free radicals and production of leukotrienes are all increased.^[18] The product of the 5-lipoxygenase pathway may also play a key role in the development of ulcer induced by irritant agents such as ethanol. In the present study also the significant protection exhibited by the test extract against ethanol induced gastric ulceration may be due to inhibition of 5-lipoxygenase pathway or leukotriene antagonistic activity. Flavonoids have also been reported to offer some protection in ulcer development by increasing capillary resistance and improving microcirculation.^[19] Hence the gastro protective property may be assigned to the antioxidant principles like flavonoids and tannins which are present in the leaves of the *Spathodea campanulata* P.beauv.

CONCLUSION

The results of the present investigation illustrated that ethanolic extract of the plant produced significant protection over ethanol induced gastric ulceration. Thus, ethanolic extract of leaves of *Spathodea campanulata* P.beauv. exhibited significant gastroprotective activity in rats.

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