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Antiulcer and Anti-inflammatory Activity of Aerial Parts *Enicostemma littorale* Blume

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ABSTRACT

The antiulcer and *in vitro* anti-inflammatory activities of the aerial parts of *Enicostemma littorale* against aspirin, ethanol, and pyloric ligation-induced ulcers in rats and bovine serum albumin denaturation were studied. The extract (200 mg/kg and 400 mg/kg po) was administered to the overnight fasted rats, one hour prior to aspirin / alcohol / pyloric ligation challenge. The ulcer index, tissue GSH levels, and lipid peroxidation levels were estimated in all the models of ulcers and the volume of gastric secretion, acidity, and pH, were estimated in the pyloric ligation model of ulcers. Pretreatment with the extract showed a dose-dependent decrease in the ulcer index (Against Aspirin, ethanol challenge, and pyloric ligation. The prior administration of the extract also reduced the total acidity, free acidity, and volume of gastric secretion, and elevated the gastric pH. In addition, it was also observed that the extract inhibited the serum albumin denaturation in a dose-dependent manner. It may be concluded that the methanolic extract possesses antiulcer activity, and the anti-inflammatory activity of the extract may be attributed to the antioxidant potential, as reported earlier.

Key words: Anti-inflammatory, antioxidants, antiulcer, *Enicostemma littorale*

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INTRODUCTION

Various mechanisms have been suggested for explaining the pathogenesis of peptic ulcer, which results due to an imbalance between the protective mechanisms and aggressive factors such as pepsin and acid.[1] In addition, the free radicals involved in tissue damage have been reported to play a role in the causation of gastric ulcers. Several classes of drugs are being adopted in the treatment of ulcers to restore the balance between aggressive and protective factors involved in the causation of ulcers. However, administration of drugs for a prolonged period is required to treat ulcers. Prolonged usage of such synthetic agents may cause side effects / drug interactions with concomitantly used drugs or food. It is unaffordable for common man to take such drugs for a prolonged period due to their escalating costs. Therefore, a large section of the world's population relies on traditional remedies / alternative systems of medicines to treat a plethora of diseases including gastric ulcers.[2]

Hence, in our search for herbal remedies for diseases that require chronic treatment, we found that *Enicostemma littorale* Blume a glorious perennial herb belonging to the family Gentianceae. Upon literature survey it was found that the hot extract from it is being used by tribal healers of interior Gujarat, for the treatment of diabetes, fever, stomach ache, dyspepsia, and malaria. It is also reported

to possess antitumor,[3] antiarthritic,[4] hypoglycemic,[5] and antimalarial activities.[6] There are reports that the plant possesses flavonoids, xanthines, and so on, in the aerial parts of this plant.[7] The flavonoids are known to have antioxidant, antiulcer, and anti-inflammatory properties. However, there are no reports on the gastroprotective activity of the plant. As, this plant is reported to contain flavonoids and other related compounds, the methanolic extract of this plant is investigated for gastroprotective and *in vitro* anti-inflammatory activities by using various experimental models of ulcers namely pyloric ligation, ethanol, and aspirin-induced ulcers in albino rats.

MATERIALS AND METHODS

Collection of plant material

The shoots of plant *Enicostemma littorale* Blume were collected from Karnataka (India) from August to September, 2005) at the end of flowering season and were authenticated By Prof. K. Prabhu, Department of Pharmacognosy, S. C. S. College of Pharmacy, Harapanahalli. The voucher specimen was deposited at SCSCOP, Harapanahalli.

Preparation of the extract

The plant material was dried under shade. The shade-dried material was powdered. The coarse powder was subjected to successive extraction using solvents with increasing order of polarity that is, petroleum ether, chloroform, and methanol, and macerated with chloroform water.[8] All the extracts were subjected to the preliminary phytochemical tests and the methanolic extract showed the presence of flavonoids. Hence, the methanolic extract was selected for further studies.

Animals

Albino rats (125 – 175 g) and mice (18 - 25 g) of either sex were obtained from NIMHANS, Bangalore, and were kept in standard plastic animal cages in a group of six to eight in each cage, at standard conditions, with 12 hours of light and dark cycle, in an institutional animal house. The animals were fed with the standard rodent diet and with water *ad libitum*. After one week of acclimatization the animals were used for further experiments. The CPCSEA approval number was Reg. No.157/1999/CPCSEA. Approval from the institutional animal ethical committee for the usage of animals in the experiments and experimental protocol was obtained as per the Indian CPCSEA guidelines for the usage of laboratory animals prior to the experimentation.

Acute toxicity studies

The extracted methanol was tested for acute toxicity studies as per CPCSEA guideline No. 420. No animals died even at 2000 mg/kg and hence one-tenth and one-fifth of 2000 mg/kg was selected for further investigations.

Anti ulcer activity

Aspirin (ASP)-induced ulcer[9]

Albino rats of either sex weighing between 180 and 200 g were divided into five groups of six animals each and fasted for 24 hours with water *ad libitum,* prior to the experiment. The animals of groups 1 and 2 were pre-treated with vehicle and the animals of group 3 were treated with standard, that is, lansoprazole 8 mg/kg. Similarly the animals of groups 4 and 5 were pre-treated with methanolic extract 200 mg/kg and 400 mg/kg, respectively. Aspirin (200 mg/kg p o) was administered to the animals of groups 2 – 5, 60 minutes after the respective treatments. The animals were then sacrificed by cervical dislocation after six hours. The stomach was taken out and cut open along the greater curvature. The number of ulcers per stomach were noted and severity of the ulcers were observed microscopically and scoring was done as follows:[10] Zero for normal colored stomach, 0.5 for red coloration, 1 for spot ulcer, 1.5 for hemorrhagic streaks, 2 for ulcer > 3 but < 5 mm, and 3 for ulcer > 5 mm. The mean ulcer score for each animal was expressed as the ulcer index. The percentage protection was calculated.

Ethanol induced (EtoH)-induced ulcer[11]

Albino rats of either sex weighing between 180 – 200 g were divided into five groups of six animals each and fasted for 24 hours with water *ad libitum,* prior to the experiment. The animals of groups 1 and 2 were pre-treated with vehicle and the animals of group 3 were treated with standard, that is, lansoprazole 8 mg/kg. Similarly the animals of groups 4 and 5 were pre-treated with methanolic extract 200 mg/kg and 400 mg/kg, respectively. Ethanol (100% 1 ml/200 g, p o) was administered to all the animals of groups 2 – 5, 60 minutes after the respective treatments. The animals were sacrificed by cervical dislocation after one hour of EtOH administration and the stomach was incised along the greater curvature and examined for ulcers. The ulcer index was scored as mentioned earlier, using Kulkarni's method,[10] and the percentage protection was also reported.

Pylorus-ligated (PL) rats[12]

Albino rats of either sex weighing between 180 – 220 g were divided into five groups of six animals each, fasted for 18 hours and care was taken to avoid coprophagy.