

ANTIULCEROGENIC EFFECT OF ETHANOL EXTRACT OF *ENICOSTEMMA LITTORALE* LINN.

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ABSTRACT

Ulcers are caused by an imbalance between offensive factors like gastric acid, pepsin and the defensive factors like mucus, HCO₃⁻, increase in pH and mucosal blood flow. Present work reports the potent antiulcer activity of *Enicostemma littorale* by reducing offensive factors and increasing the defensive factors in pylorus ligated rats. The extract was found to be significant when compared to control. Ranitidine was taken as reference drug.

KEY WORDS: *Enicostemma littorale*, antiulcer, ethanol extract.

1. INTRODUCTION

Peptic ulcer is one of the most frequent disorders of the alimentary tract and in various countries its prevalence is estimated as 5-10% of the adult population. This disorder remains one of the most important problems, both in the practice of primary health care physicians and gastroenterologists (Schabowski, 2004). Ulcers are caused by an imbalance between aggressive factors (gastric acid, pepsin, *Helicobacter pylori*) and the defensive factors (mucus, HCO₃⁻ and mucosal blood flow) (Takeuchi Koji, 1999). Factors which are responsible for pathogenesis of ulcers are increased basal secretory drive, increased parietal cell mass, increased post prandial acid secretion, increased post prandial gastrin release, increased sensitivity of secretagogues, decreased inhibition of acid and gastrin secretion, rapid delayed gastric emptying and decreased mucosal resistance. Genetic and environmental factors are also thought to play a role (Feldman, 2000).

Peptic ulcers occur in the region of duodenum and stomach. They extend through the muscularis mucosa into the submucosa or deeper into stomach (Cotran, Kumar, 2001). The complications of ulcers are commonly hemorrhage, mucosal erosions, perforation, obstruction and malignant transformations (Rang, 2003).

Enicostemma littorale Linn, is a belonging to family *Gentianaceae*, is commonly known as chota chirayta (Kirtikar, Basu, 1935). It is a shrub found throughout India up to 1.5 feet height and more frequently near the sea (Kavimani, Manisenthkumar, 2000). The plant as a whole has been used as antidiabetic, swelling, itches and for anti-cancer diseases from ancient times (Kirtikar, 1935). In ayurveda the plant is used to treat various gastrointestinal disorders (Kavimani, 2000). In our previous research work we have reported analgesic activity of the plant (Shivakumar, 2008). However, no scientific study on antiulcer property of *Enicostemma littorale* plant has been reported.

The plant has been reported to contain flavonoids, steroids, Glycoside and alkaloids (Vasu, 2003). It was found from the result of review literature that, the drug which contain active constituents like flavonoids possess antiulcer activity (Surana, 2008).

In the present study, we aimed at evaluating the under utilized and easily available plant for antiulcer activity in pylorus ligated rats.

2. MATERIALS AND METHODS

Animals:

Adult albino rats weighing 180-200 g were used for the experiment. The animals were housed in polypropylene cages at 24 ± 2°C and were fed with proper food and water *ad libitum*. The animals were divided into 3 groups of 6 animals each. The ethical clearance was obtained by the institutional animal ethics committee (Registration No. 126/CPCSEA) before experiment.

Materials:

Topfer's Reagent, Folin-ciocalteu's reagent, Trichloroacetic acid, Bovine albumin, Alcian blue 8 GX,

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other chemicals and reagents were purchased from S.D. Fine Chemicals, Mumbai and were of analytical grade.

The plant of *Enicostemma littorale* were collected from near by areas of Harapanahalli, Davanageri district in the month of October and November and were authenticated by Prof. M.B.Mamadapur, Dept.of Botany, J.T.College, Gadag. A voucher specimen [KLECPG/02/2007] has been deposited at the departmental herbarium for future purpose.

Preparation of extracts:

The dried plant leaves approximately (500 gm) were comminuted to coarse particle size no. (#) 40 and subjected to continuous hot extraction with 90 % ethanol in a soxhlet extractor for 48 hr. The total ethanol extract was filtered and concentrated to dryness at 40° C under reduced pressure in a rota evaporator. The yield of ethanol extract was found to be 100 gm (20 % w/w). The extract was kept in a dessicator till the experiment.

Acute Toxicity Evaluation (LD₅₀):

The acute toxicity of *Enicostemma littorale* was determined in female albino mice. The animals were fasted overnight prior to the experiment and fixed dose method of Committee for the Purpose of Control and Supervision on Experimental Animals. Guideline No.420 was adopted .

Pyloric Ligation Model:

In this method albino rats were fasted in individual cages for 24 hr. Care was being taken to avoid Coprophagy. Control vehicle, ethanol extract and reference drug (Ranitidine) were administered by oral route. The pyloric ligation was carried out 30 min and 4 hr after the drug administration in each group animals. Under light ether anesthesia, the abdomen was opened and the pylorus was ligated. The abdomen was then sutured. After 4 hr of pyloric ligation, the animals were sacrificed with excess of anesthetic ether and the stomach was dissected out. Gastric juice was collected and its volume, pH and total acidity was measured. Ulcer index was determined. The stomach was opened along the greater curvature and the severity of hemorrhagic erosions in the acid secreting mucosa was assessed on a scale of 0 to 3 as given below (Komarov ,1945)

Normal color – 0, Red color – 0.5, Red spots – 1, Hemorrhagic streaks – 1.5, 3 > 5 ulcers – 2 and < 5 ulcers – 3.

Mean ulcer score for each animal is expressed as ulcer index. The percentage protection was calculated using the formula.

Percentage of Ulcer protection = $U_t / U_c \times 100$

Where U_t = ulcer index of treated group and

U_c = ulcer index of the control group

In order to calculate the difference between the control and the treated animals the results were subjected to student 't' test.

Gastric juice was collected from pylorus ligated rats. The gastric juice thus collected was centrifuged and the volume of gastric juice as well as pH of gastric juice was noted. The gastric juice was subjected to biochemical estimations as follows:

Determination of free and total acidity:

One ml of gastric juice was pipetted into a 100 ml conical flask, added 2 or 3 drops of Topfer's reagent and titrated with 0.01N Sodium hydroxide until all traces of red colour disappears and the colour of the solution was yellowish orange. The volume of alkali added was noted. This volume corresponds to free acidity. Then 2 or 3 drops of phenolphthalein solution was added and titration was continued until a definite red tinge appears. Again the total volume of alkali added was noted. The volume corresponds to total acidity (Hwak ,1974). Acidity was calculated by using the formula

$$\text{Acidity} = \frac{\text{Volume of NaOH} \times \text{Normality of NaOH} \times 100}{0.1} \text{ mcq/lit/100gm}$$

The statistical significance was determined by using student 't' test.

Estimation of total proteins:

The dissolved proteins in gastric juice was estimated in the alcoholic precipitate obtained by adding 90 % alcohol with gastric juice in 9:1 ratio respectively. Then 0.1 ml of alcoholic precipitate of gastric juice was dissolved in 1ml of 0.1N NaOH and from this 0.05 ml was taken in another test tube, to this 4 ml of alkaline mixture was added and kept for 10 min. Then 0.4 ml of phenol reagent was added and again 10 min was allowed for colour development. Reading was taken against blank prepared with distilled water at 610 nanometer in Hitachi 15-20 spectrophotometer. The protein content was calculated from the standard curve prepared with bovine albumin and has been expressed in terms of mcg/ml of gastric juice (Lowry ,1951). The statistical analysis was calculated by using student 't' test.

Estimation of mucin:

After the collection of gastric juice, the glandular portions excised and opened down the lesser curvature. The everted stomachs were soaked for 2 hr in 0.1 % alcian blue 8 GX dissolved in 0.16 M sucrose buffered with 0.05 M sodium acetate adjusted to a pH with HCl. Uncomplexed dye was removed by 2 successive washes of 15 and 45 min in 0.25M sucrose solution. Dye complex with mucus was diluted by immersion in 10 ml aliquots of 0.5 M magnesium chloride for 2 hr. The resulting blue solutions were shaken briefly with equal volume of diethyl ether and optical density of the aqueous phase was measured at 605 nm in Hitachi 15-20 spectrophotometer. The mucin content of the sample was determined from the standard curve of mucin has been expressed in mcg/gm of wet gland tissue (Corne, 1974).

Estimation of pepsin:

From each determination place 4 tubes (1) and (2) containing 5 ml of substrate, (3) and (4) containing 10 ml of TCA in the water bath at 37 °C. The gastric juice was mixed with equal volume of Hydrochloric acid at pH 2.1, warmed to 37 °C added 1 ml of mixture to each of tubes 1 and 4. Incubated for 15 min at the end of which time mix tube 1 with tube 3. Allowed to stand both for about 4 min. 1+3 gives test and 2+4 gives blank. Filtered, 25 to 30 min after the beginning of the filtration, 2 ml of filtrate was pipetted into 10 ml of NaOH. Mixed by gentle rotation, then 1 ml of phenol reagent was added and again mixed by gentle rotation. After 30 min the intensity of the colour was measured at 680 nm in Hitachi 15-20 spectrophotometer. The difference between test and blank gives the measures of peptic activity. As standard, mixed 2 ml of freshly prepared phenol solution containing 50 µg/ml with 10 ml NaOH and 1 ml of phenol reagent was added and was measured at 680 nm after 5 to 10 min (Debnath, 1974).

3. RESULTS AND DISCUSSION

The acute toxicity studies of *Enicostemma littorale* was found to be safe and no mortality of the animals was observed at the of 2000 mg/kg. Hence, 2500 mg/kg was fixed as LD₅₀ cut off value for extracts. So that 1/10th of the extract was selected for the evaluation of antiulcer. i.e. 250 mg/kg.

It is evident from the Table No.1 that, the decrease in ulcer index by ranitidine and *Enicostemma*

littorale. Ranitidine showed decrease in the volume of gastric juice, free acidity and total acidity when compared to control which are statistically significant. While, *Enicostemma littorale* also shown decrease in volume of gastric juice, free acidity and total acidity that are also statistically significant. In pH also a significant difference was observed between *Enicostemma littorale* treated and control animals.

A significant difference in the mucin content, total proteins and pepsin content (Table No. 2) was observed in rats treated with *Enicostemma littorale* in comparison to control.

In pyloric ligation model, the significant reduction in basal gastric secretion and complete inhibition of ulcers by *Enicostemma littorale* after pylorus ligation suggest that the cytoprotective mechanism of action of the *Enicostemma littorale* on gastric mucosa may responsible for direct reduction of gastric secretion through one or more of the possible mechanisms discussed by Parmar (1983), Martin (1993) and Njar (1995). Moreover, gastric acid is an important factor for the genesis of ulceration of pylorus ligation ulcer in rats (Komarov, 1945). Gastric acid secretion is regulated by many factors including anxiotic effect in the CNS, vagal activity, cholinergic, histaminergic and gastrinergic neurotransmissions, the activities of various post-synaptic receptors and the proton pump. It is therefore, difficult to elucidate the relationship between the mechanisms of inhibition of gastric acid by *Enicostemma littorale*. The current data clearly demonstrated that *Enicostemma littorale* inhibited the aggressive factor, gastric acid secretion. The anti-ulcerogenic effect of the *Enicostemma littorale* may be related to its anti-secretory action since acid is a major factor in the development of peptic ulcer (Glavin, 1992). The decrease in the protein content of gastric juice by *Enicostemma littorale* suggests that, there is a decrease in leakage of plasma proteins into gastric juice. However, certain anti-ulcer drugs increase the amount of gastric mucus secretion in the gastric mucosa (Bolten, 1976 and Robert, 1984). This mucus consists of mucin-type glycoproteins, which can be detected by amounts of alcian blue binding (Bolten, 1978). *Enicostemma littorale* increased the alcian blue binding to mucosa. The increase in bound alcian blue suggested protective effect of orally administered *Enicostemma littorale*. This may be via the formation of protecting complexes

between *Enicostemma littorale* and mucus, which can act as a barrier against several agents, introduced in the stomach (Clamp, 1978; Sun, 1991). Thus, the possible mechanism of gastric mucosal protection by orally administered *Enicostemma littorale* may be partly due to either decrease in proteins or reinforcement of resistance of the mucosal barrier by a protective coating. In addition, its anti-secretory activity cannot be excluded.

The anti-ulcer *Enicostemma littorale* was supported by the decreases in the aggressive factors like basal gastric acid secretion, total acidity, total proteins and pepsin content and an increase in defensive factors like pH and mucin content. However, the mechanism of its ulcer healing activity needs to be explored experimentally. Further clinical studies may help in extrapolating the result of present study to human beings.

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Table 1: Effect of *Enicostemma littorale* on Ulcer index, Volume of gastric juice, pH, Free acidity and Total acidity.

S.No	Treatment juice/100 g	Ulcer index	Volume of gastric (ml)	pH	Free acidity (meq/l/100 g)	Total acidity (meq/l/100 g)
1	Control	4.83 ± 0.45	3.24 ± 0.28	1.73 ± 0.11	21.83 ± 1.22	84.83 ± 2.11
2	Ranitidine	1.41 ± 0.16*	1.02 ± 0.08*	3.21 ± 0.29*	07.00 ± 0.42*	41.83 ± 1.66*
3	<i>Enicostemma littorale</i>	1.53 ± 0.23*	1.16 ± 0.09*	3.13 ± 0.27*	07.40 ± 0.57*	42.50 ± 1.67*

*indicates significantly different from control, P < 0.01. All values are represented as mean ± SEM (n=6)

Table 2: Effect of *Enicostemma littorale* on Protein content, Pepsin content and Gastric wall mucus content

S.No	Treatment	Total Protein content (µg/ml)	Pepsin activity (µmole/ml)	Gastric wall mucus content (µg of alcian blue/g of wet tissue)
1	Control	115.64 ± 2.22	46.41 ± 1.27	252.21 ± 2.86
2	Ranitidine	111.56 ± 1.86*	25.65 ± 0.86**	284.31 ± 2.93**
3	<i>Enicostemma littorale</i>	58.50 ± 1.40*	23.44 ± 0.78**	307.90 ± 3.10**

* indicates significantly different from control * P < 0.05, ** P < 0.01, All values are represented as mean ± SEM. (n=6).

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