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Evaluation of Anti-Ulcer and Anti-Secretory Activity of Hybanthus Enneaspermus

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Abstract

The major causes of ulceration is due to hyper secretion of gastric juice and pepsin which is due to lack of equilibrium between the gastric aggressive factor and mucosal defense factor .The objective of the present study was to investigate the anti ulcer and anti secretory activity of alcoholic and aqueous extract of whole plant *Hybanthus enneaspermus* Muell (violaceae). The percentage of yield was found to be 14.6 % and 12.9 % for alcoholic and aqueous extracts respectively. Anti-ulcer effect of both extracts (100 mg/kg) were evaluated in rats using pyloric ligation (PL) induced gastric ulcer model compared with ranitidine in terms of inhibition of release of gastric juice, hydrochloric acid and neutralization activity. The observation was made for ulcer sores, ulcer index, free acidity, total acidity and pH. At the end of the study the alcoholic extract (100mg/kg) exhibited more potency then aqueous extract which was found to be significant compared to standard drug ranitidine having the property to reduce gastric acid secretion, reduction in ulcer index and induction of neutralizing activity. The study concludes that aqueous and alcoholic extract of *Hybanthus enneaspermus* possess potentially useful anti-ulcer activity in pylorus ligated model in rats.

Key Words: *Hybanthus enneaspermus, Anti* ulcer activity, Ulcer Index**.** Topfer reagent

Introduction

Gastric ulcers are mucosal lesions that result from an imbalance between aggressive factors such as acid and pepsin, and defensive mechanisms like gastric mucous, high mucosal blood flow and high mucosal turnover rate that work towards maintenance of mucosal integrity [1]. Another factor that has been implicated in the pathogenesis of gastric ulcers is oxidative stress in the gastric mucosa [2]. *Hybanthus enneaspermus* Muell belongs to family Violaceae is an herb or a shrub distributed in the tropical and subtropical regions of world. It is an herb often with woody troches found in the warmer parts of India. The plant is popularly known as *Ratanpurus* (Hindi). Traditionally the plant is used as an aphrodisiac, demulcent, tonic, diuretic, in urinary infections, diarrhoea, leucorrhoea, dysuria, in inflammation and sterility [3] an infusion of the plant extract is given in case of cholera. The plant has been reported to have anti-inflammatory, antitussive, antiplasmodial, antimicrobial [4] anti convulsant and freeradical scavenging activity. The plant is reported to contain aurantiamide acetate, isoaborinol, sitosterol and triterpene [5]. In folklore the plant is used in case of gonorrhea, urinary infections and in inflammatory conditions. By considering the free radical scavenging activity the present study aims for antiulcer and antisecretory evaluation the plant extract with pyloric ligation rat model.

Materials and Methods

Collection of plant material

The plant was collected from rural belt of Bhubaneswar, Orissa and was authenticated in the

department of Botany, Utkal University, Bhubaneswar. The plant was collected in bulk and washed with tap water to remove the soil and dirt particles and then shade dried. The dried plant materials were milled into coarse powder by a mechanical grinder and sieved in sieve 20. The coarse powder was taken for extraction in soxhlet apparatus and fine powder for maceration.

Preparation of extract

Alcoholic Extract

The powdered plant (2.5 kg) was exhausting extracted by soxhlet apparatus with 95% ethanol. The total ethanol extract was then concentrated in vacuum to syrupy mass.

Aqueous Extract

The powdered plant material (25 kg) was macerated with chloroform water (1:9) for seven days. The extract was filtered and concentrated over a water bath and further dried in vacuums oven till constant weight

Experimental animals

Adult male albino mice 20 – 25 gm and rats 150 – 200 gms were used for the study. Animals were kept in the animal house of GIET School of Pharmacy, Rajahmundry, maintained under standard husbandry condition with free access to food and water *ad libitum*. All the experiments in this study were approved by institutional animal ethical committee with CPCSEA registration number 1069/PO/ac/07/CPCSEA, GIET School of pharmacy

Acute toxicity studies

Oral acute toxicity studies were carried out with Albino mice weighing 20 – 25 gm, with 2 mice per dose group. The extracts were administered as per the staircase method [6]. The mice were fed with alcoholic and aqueous extract of *H. enneaspermus* separately suspended in 2% of gum acacia at dose 1000, 2000, 3000, 4000, 5000 mg/kg bodyweight. The animals were observed continuously for 2 h for the gross behavioral changes and then intermittently once in every 2 h and finally at the end of 24 and 72 h to note for any signs of toxicity including death.

Pyloric Ligation Induced Gastric Ulcer Method

Albino Rats were weighing between 100-200gms were fasted for 24hrs but were allowed free access to water. Under light ether anesthesia, the pylorus was ligated through a midline abdominal incision with care not to damage any blood vessels. After wound closure, the animals were caged individually without food and water during this period. At the end of experiment, the animals were sacrificed by euthanasia and the stomach was removed. Gastric contents were drained from the stomach and centrifuged at 3000 rpm for 5 min, and the volume of supernatant solution was measured. Free and total acidity were estimated by titration with 0.1 N NaOH using topfer reagent and phenolphthalein as indicator. Acid output was expressed in mEq by multiplying the volume in ml by the acid concentration in mEq/l [7].

Determination of Free Acidity of Gastric Juice

One milliliter of the supernatant liquid was pipetted out and diluted to 10 ml with distilled water. The solution was titrated against 0.01N NaOH solution using topfer's reagent as indicator, to the end point the solution turned to orange color. The volume of NaOH needed was taken as corresponding to the free acidity. Acidity was determined by using

Acidity=

$$\frac{\text{volume of NAOH x normality x 100mEq/L/100gm}}{0.1}$$

Determination of Total Acidity of Gastric Juice

An aliquot of 1 ml of gastric juice was taken in to a 50 ml conical flask and two drops of phenolphthalein indicator was added and titrated with 0.01N NaOH until a permanent pink color was established .The volume of 0.01N NaOH consumed was noted, total acidity was calculated and expressed as mEq/l.

Determination of pH

The pH of the gastric juice was determined by using pen type pH meter.

Measurement of Ulcer Index

Immediately after the animals were sacrificed, their stomach was dissected out, incised along the greater curvature and the mucosa was rinsed with cold normal saline to remove blood contaminant, ulcers were examined under a magnifying lens. The ulcers were measured with the help of vernier caliper using the following arbitrary scale.

The scale was as follows, if,

Score 0 = no ulcers; Normal stomach,

Score 0.5 = red coloration

Score 1 =spot ulcer; petechial hemorrhage,

Score 1.5 = hemorrhagic

Score 2 = ulcers<2mm,

Score 3 = ulcers>2<4; perforation

Score 4 =ulcers > 4mm

Results & Discussion

From the above study it was indicated that Reduction in volume of gastric juice was significant (P < 0.001) compared to control for alcoholic extract(100mg/kg) indicating their good antisecretory potential. The acid neutralizing capacity as indicated by pH of the gastric fluid was found to be more for alcoholic extract (100mg/kg) as compared to standard drug ranitidine. This extract also reduces the free acidity and total acidity as compared to ranitidine. From table-1 it concludes that all the extracts produced a dose dependent and significant (P<0.01) reduction in the ulcer index.

Conclusion

Various factors that have been implicated in the pathogenesis of gastric ulcers are an increase in gastric acid secretion, pepsin activity and oxidative stress in the gastric mucosa. In conclusion, our results showed that the anti ulcer and anti secretory activity of alcoholic and aqueous extract of whole plant *Hybanthus enneaspermus* Muell suggest the potential to exhibit anti-ulcer properties owing to their phytochemical constituents which either induce antioxidant enzymes or directly contribute to free-radical scavenging activity.

TABLE-1: Anti-Ulcer and Anti-Secretory Activity of *Hybanthus Enneaspermus*

Group	Volume(ml)	pH	Ulcer index	Free acidity (mEq/l)	Total acidity (mEq./l)	% Protection
Control	6.4±0.3	1.91±0.26	16.8±1.15	46.17±3.6	50.38±2.5	……..
Ranitidine (50mg/kg)	2.16±0.28**	5.05±0.33**	2.75±0.42*	6.86±0.58*	8.15±0.462*	83.60
E.extract (25mg/kg)	4.81±0.36	2.38±0.27	6.8±0.41**	31.0±4.01	36.3±3.6	59.02
E.extract (50mg/kg)	3.9±0.54	2.90±0.37	6.28±0.42**	32.17±3.89	39.0±3.83	62.60
E.extract (100mg/kg)	2.3±0.25**	4.1±0.15**	4.2±0.28**	8.81±0.88**	15.8±1.8**	74.70
Aq. extract (25mg/kg)	4.81±0.36	2.38±0.27	6.83±0.41**	31.0±4.01	36.33±3.62	59.02
Aq. extract (50mg/kg)	3.23±0.42*	3.24±0.28	6.4±0.38*	36.50±3.58	42.17±3.46	61.90
Aq. extract (100mg/kg)	4.7±0.25	3.3±0.25	6.08±0.50*	38.3±4.29	42.0±4.18	63.79

Data are represented as mean±S.E.M. Statistical analysis was done with one way analysis of variance (ANOVA). ** p<0.001,* p<0.05 as compared to control (n=6 in each group).

"Cite this article"

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