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ANTI ULCER ACTIVITY OF *HELIOTRPIUM INDICUM* LEAVES EXTRACT

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ABSTRACT

The objective of present study is to evaluate the anti ulcer activity of ethanol extract of leaves of *Heliotropium indicum*. The ethanol extract of *H. indicum* was investigated for its anti ulcer activity against Aspirin plus pylorus ligation induced gastric ulcer in rats, HCl- Ethanol induced ulcer in mice and water immersion stress induced ulcer in rats. The antiulcer activity was assessed by determining and comparing gastric volume, free acidity and ulcer inhibition in aspirin plus pylorus ligation induced gastric ulcer model. The number of lesions in HCI-Ethanol induced peptic ulcer model and mean score value of ulcer inhibition in water immersion stress induced ulcer model. A significant antiulcer activity of plant extract was observed in all the models. Pylorus ligation model showed significant reduction in gastric volume, free acidity and ulcer index as compared to control. Also extract showed significant ulcer inhibition in HCl- Ethanol induced ulcer and ulcer protection index in stress induced ulcer. This present study indicates that *Heliotropium indicum* leaves extract have potential anti ulcer activity in the three models tested.

INTRODUCTION: Gastric ulcers one of the most widespread diseases is believed to be due to an imbalance between aggressive and protective factors. The gastric mucosa is continuously exposed to potentially injurious agents such as acid, pepsin, bile acids, food ingredients, bacterial products (*Helicobacter pylori*) and drugs. These agents have been implicated in the pathogenesis of gastric ulcer, including enhanced gastric acid and pepsin secretion, inhibition of prostaglandin synthesis and cell proliferation growth, diminished gastric blood flow and gastric motility [1].

Drug treatment of peptic ulcers is targeted at either counteracting aggressive factors (acid, pepsin, active oxidants, platelet aggravating factor "PAF," leukotrienes, endothelins, bile or exogenous factors including NSAIDs) or stimulating the mucosal defenses (mucus, bicarbonate, normal blood flow, prostaglandins, nitric oxide [2]. The goals of treating peptic ulcer disease are to relieve pain, heal the ulcer and prevent ulcer recurrence. Currently, there is no cost-effective treatment that meets all these goals. Hence, efforts are on to find a suitable treatment from natural product sources. A large number of spices and herbs have been evaluated by various researchers for their anti-ulcer effects to achieve a favorable outcome [3].

Heliotropium indicum (Boraginaceae) - commonly called as Indian Turnsole, is a herb with slightly woody at base. It is distributed in the tropical and temperate regions of the world and found throughout India [4]. The whole plant is claimed to possess medicinal properties. In ayurveda the juice of leaves applied on boils, pimples, ulcers, sores and wounds to cure. The plant is used for diarrhea, malaise or vomiting in infants. The leaves are used for the treatment of ophthalmic disorders, erysipelas, pharyngodynia, anti-tumor and anti-inflammatory. The roots are used as astringent, expectorant and febrifuge. The extract of leaves was proved to be active against Schwart's leukemia [5].

MATERIALS AND METHODS:

Plant Material: The leaves of *H.indicum* were collected from Udupi, Karnataka, during October 2009. It was authenticated by Department of Botany, Poorna Prajna College, Udupi, Karnataka, India. A voucher specimen (HI.119) was deposited in the herbarium of our Institute.

Preparation of Extract: Leaves were shade dried and powdered mechanically. The powder was loaded into Soxhlet extractor in 8 batches of 250 g each and was subjected to extraction for about 30- 40 h with 95% ethanol. After extraction the solvent was distilled off and the extract was concentrated under reduced pressure using a rotary flash evaporator (Buchi, Flawil, Switzerland) to a syrupy consistency. Then it was dried in the dessicator.

Animals: Twelve week-old healthy Wistar rats (150-200 g) of either sex procured from Indian Institute of Sciences Bangalore were used for this study. They are maintained under standard conditions (temperature 22±2^{o}C, relative humidity 60±5% and 12 h light/dark cycle).The animals were housed in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free access to standard pellet diet and water *ad libitum*. Experiments were conducted between 9: 00 to14: 00 h. Each rat was used only once. The Institutional Animal Ethics Committee approved the experimental protocol. All the animals received humane care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the "National Academy of Sciences" and published by the "National Institute of Health".

Drugs: The reference drugs such as Ranitidine, Sucralfate, Omeprazole and the test extract of *H.indicum,* were suspended in 1% sodium carboxy methyl cellulose (SCMC) and used for anti-ulcer studies. Each drug suspension was prepared freshly just before the administration. Drugs and vehicles were administered orally.

The present study followed three approaches of antiulcerogenic mechanism of the plant extract:

1. Aspirin plus pylorus ligation induced gastric ulcer in rats (antisecretory mechanism).
2. HCl- Ethanol induced ulcer in mice (cytoprotective mechanism).
3. Water immersion stress induced ulcer in rats (proton pump inhibition mechanism).

Aspirin plus pylorus ligation induced gastric ulcer in rats: The rats were divided into 3 groups (n=6). All the animals received 200 mg/kg of aspirin once daily for three days. The different groups of animals are assigned as follows.

Group I (control): received 1ml/kg, 1% SCMC.

Group II (reference standard): treated with 50 mg/kg Ranitidine.

Group III treated with 500 mg/kg ethanol extract of *H. indicum*.

On the fourth day pylorus part was ligated following 36 hour fasting [6]. Four hours after the pyloric ligation the animals were sacrificed by decapitation. The stomach was opened and the ulcer index was determined [7]. The gastric content was titrated against 0.01 N NaOH to find out the free acidity and total acidity [8].

Ulcer lesion Index method: HCl - Ethanol induced ulcer: Swiss albino mice were divided into 3 groups (n=6). The different groups of animals are assigned as follows.

Group I (control) received 1ml/kg 1% SCMC.

Group II (reference standard) received 100 mg/kg Sucralfate.

Group III received 500 mg/kg ethanol extract of *H. indicum*.

After 1 hour all the animals were treated with 0.2 ml of HCl - Ethanol mixture p.o (0.3 M Hydrochloric acid and 60% ethanol) to induce gastric ulcer. After 1 hour, animals were sacrificed by cervical dislocation. The stomach was excised and lesion index was determined by measuring each lesion in mm along its greater length [9].

Water immersion stress induced ulcer in rats: Stress ulcers were induced by forced swimming in the glass cylinder (height 45 cm, diameter 25 cm) containing water to the height of 35 cm maintained at 25°C for 3h [10]. Rats were fasted for 24h prior to the experiment and divided in to 3 groups (n=6). The different groups of animals are assigned as follows.

Group I (control), received 1 ml/kg 1% SCMC.

Group II (reference standard) treated with 20 mg/kg Omeprazole.

Group III received 500 mg/kg ethanol extract of *H. indicum*.

After the drug treatment animals were allowed to swim in water for 3 hour. The stomach of each animal was removed and the extent of gastric damage was assessed [11].

Statistical Analysis: The statistical analysis of all the results was carried out using one way ANOVA followed by Dunnett's multiple comparisons using graph pad in stat 3 and all the results obtained in the study were compared with the vehicle control group.

RESULTS: In aspirin plus pylorus ligation induced gastric ulcer model, the ethanol extract of *H. Indicum* showed significant reduction in gastric volume, free acidity and ulcer score as compared to control.

TABLE 1: EFFECT OF *H. INDICUM* ON ASPIRIN PLUS PYLORUS LIGATION INDUCED GASTRIC ULCER IN RATS

Treatment (mg/kg)	Volume of gastric secretion (ml/100g)	Free acidity (mEq/100g)	Total acidity (mEq/100g)	pH
Control (1% SCMC)	2.633±0.042	225.00±6.124	555.00±7.50	2.20±0.163
Ranitidine (50mg/kg)	1.317±0.172*	148.75±13. 475#	492.5±20.736*	3.167±0.166*
H. indicum (500mg/kg)	1.675±0.083*	152±10.782#	548.5±10.724	2.311±0.210

Values are expressed as mean ± SEM. n=6. *P<0.05, #P<0.01 as compared to control

TABLE 2: EFFECT OF *H. INDICUM* ON ASPIRIN PLUS PYLORUS LIGATION INDUCED GASTRIC ULCER IN RATS

Treatment (mg/kg)	Ulcer score	Ulcer inhibition (%)
Control (1% SCMC)	3.60±0.2	
Ranitidine (50mg/kg)	0.167±0.166#	95.37
H. indicum (500mg/kg)	1.667±0.307#	53.66

Values are expressed as mean ± SEM. n=6. #P<0.01 as compared to control; it can be observed that the number of lesions in HCl-Ethanol induced peptic ulcer group was significantly high and the ethanol extract of *H. indicum* pretreated group depicted marked reduction (*P* <0.01) in gastric lesion as compared to control

TABLE 3: EFFECT OF *H. INDICUM* ON HCL-ETHANOL-INDUCED LESIONS IN GASTRIC MUCOSA OF MICE

Treatment (mg/kg)	Mean Ulcer score	Ulcer inhibition (%)
Control (1% SCMC)	22.667±3.509	-
Sucralfate (100mg/kg)	1.167±0.5426*	94.85
H. indicum (500mg/kg)	8.333±5.57*	63.23

Values are expressed as mean ± SEM. n=6. *P<0.05 as compared to control; in water immersion stress induced ulcer the mean score value of ulcer inhibition was found to be very significant (*P* <0.001)

TABLE 4: EFFECT OF *H. INDICUM* ON WATER IMMERSION STRESS INDUCED ULCER IN RATS

Treatment (mg/kg)	Mean Ulcer score	Ulcer inhibition (%)
Control (1% SCMC)	143.3±12.01	-
Omeprazole (20mg/kg)	0.0±0.0$	100
H. indicum (500mg/kg)	56.66±2.10$	60.4

Values are expressed as mean ± SEM. n=6. $P<0.001 as compared to control

DISCUSSION: In aspirin plus pylorus ligation model, ulcer index parameter was used for the evaluation of anti-ulcer activity since ulcer formation is directly related to factors such as gastric volume, free and total acidity. In vehicle control animals aspirin plus pylorus ligation increased the acid secretion, which in turn caused increase in gastric volume, low pH, increased free and total acidity resulting in higher ulcer index. The extract of *H. indicum* reduced the gastric volume, free acidity, total acidity and hence ulcer index showing the anti-secretary mechanism [12]. HCl-Ethanol induced gastric damage ranging from endothelial microvascular damage to development of macroscopic gastric mucosal lesions, which is attributed mainly to the inhibition of biosynthesis of cytoprotective PG resulting in overproduction of leukotrienes and other products of the 5-lipoxygenase pathway [13]. These agents break the mucosal barrier, provoke an increase in gastric mucosal permeability to H^+ and Na^+ ions reducing the transmucosal potential difference and induce formation of erosions and ulcers. In this model *H. indicum* extract was able to produce a significant reduction of the gastric mucosal damage, indicating a probable local increase in PG synthesis [14].

Water immersion stress is one of the best model for stress induced ulcer in animals. The model provides both emotional stress as well as physiological stress to the animal. The extract showed significant (P<0.001) ulcer inhibition.

The anti ulcer effect observed in the present study might be due to a possible relationship between protection of mucosal injury, inhibition of acid secretion and the antioxidant nature of *H. indicum*. The plant extract possess antisecretory, cytoprotective and proton pump inhibition mechanism. This study indicates that *H. indicum* extract has a potential anti ulcer activity. However further study is required to isolate the active molecule responsible for the activity.

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