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Research Article

EVALUATION OF THE ANTI-ULCER ACTIVITY OF ETHANOLIC EXTRACT OF LEAVES OF *HUGONIA MYSTAX*

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ABSTRACT

The present study was designed to evaluate the antiulcer activity of ethanol extract of leaves of *Hugonia mystax* (HMEE) in pylorus ligation, ethanol and indomethacin induced models in rats HMEE was prepared and subjected to acute toxicity study as per CPCSEA guideline no. 420. Two doses *i.e.* 200 mg/kg and 400 mg/kg were selected for the further study. In pylorus ligation induced ulcer model, the parameters taken for assessing the anti-ulcer activity were gastric volume, pH, free acidity, total acidity and ulcer index. Ulcer index was also determined in ethanol and indomethacin induced ulcer models. Pretreatment with the extract has shown dose dependent decrease in ulcer index in all the experimental models of ulcers (indomethacin, ethanol and pylorus ligation induced ulcers) and also reduced the total acidity, free acidity, gastric volume and increased the pH in pylorus induced ulcer model. However, the results of gastric volume and pH were not significant with 200mg/kg dose. It is concluded from this study that HMEE possess antiulcer properties in different gastric ulcer models. The antiulcer activity of the HMEE may be attributed to the polyphenolic compounds that are present in it.

Keywords: *Hugonia mystax,* Antiulcer, Pylorus ligation, Ethanol, Indomethacin

1. INTRODUCTION

For more than a century, peptic ulcer disease has been a major cause of morbidity and mortality. Peptic ulcer occurs due to an imbalance between the aggressive (acid, pepsin and *Helicobacter pylori*) and the defensive (gastric mucus and bicarbonate secretion, prostaglandins, innate resistance of the mucosal cells) factors [1]. Although there are many products used for the treatment of gastric ulcers, most of these drugs produce several adverse reactions [2]. Hence, Herbal medicines derived from plant extracts are increasingly being utilized as reported antiulcerogenic activity of several natural drugs due to their predominant effect on mucosal defensive factors [3, 4].

Hugonia mystax, family Linaceae, is a scandent scrub and bears yellow flowers. Leaves are alternate, elliptic-obovate glabrous and penninerved [5]. Literature review mentioned that the roots are astringent, bitter, sweet, febrifuge and anthelmentic. They are useful in fevers, verminosis and vitiated conditions of *vata*, externally as a paste for inflammation [6]. Bark of the root is also employed as an antidote to poison [7]. The modern literature revealed that the plant is reported to possess Antimicrobial activity [8-10], Anti-inflammatory activity [11], *in vitro* cytotoxic effect [12], *in vitro* anthelmintic activity [13].

Preliminary phytochemicals analysis of HMEE revealed the presence of flavonoids, tannins and saponins. There are reports that flavonoids and tannins have been found to be effective against ulcer in experimental animals [14]. Hence, the present study was undertaken with the aim to assess the antiulcerogenic properties of HMEE.

2. MATERIAL AND METHODS

2.1. *Plant Material and preparation of extracts*

Hugonia mystax leaves were collected from fields of Tirupati, Andhra Pradesh. It was identified and authenticated by Dr. K. Madhava Chetty, plant taxonomist, Dept of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh. A herbarium specimen was preserved in the college herbal museum. The leaves were shade dried at room temperature and pulverized. The ethanol extract was prepared by using 70% ethanol in a soxhlet apparatus after de-fatting with petroleum

ether and chloroform. Preliminary phytochemical study exhibited the presence of saponins, tannin and flavonoids in 70% ethanol extract of *Hugonia mystax* leaves (HMEE). So, HMEE was selected for the study of antiulcer activity.

2.2. Animals

Wistar albino rats (180-220g) and mice (18-25 g) of either sex were used for the study. Approval from the institutional animal Ethical committee (1554/PO/a/11 /CPCSEA) for usage of animal in the experiment was obtained as per the Indian CPCSEA guidelines.

2.3. Acute Toxicity Studies

The acute toxicity studies were performed on albino mice as per OECD Guideline no 420 prescribed by CPCSEA.

2.4. Anti- Ulcer Activity

2.4.1. Ethanol (EtOH) induced ulcer

The albino rats of either sex weighing between 180-200 gm were divided into 4 groups of 6 animals each and fasted for 24 hrs with water *ad libitum* prior to experiment. The animals of group 1 were pretreated with vehicle and the animals of group 2 were treated with standard *i.e.* lansoprazole 8mg/kg. Similarly the animals of group 3 and 4 were pre-treated with ethanol extract 200 mg/kg and 400mg/kg respectively. Ethanol (100% 1ml/200 g, po) was administered to all the animals of all groups, 60 minutes after the respective treatments. The animals were sacrificed by cervical dislocation after one hour of EtOH administration and stomach was incised along the greater curvature and examined for ulcers [15, 16]. The number of ulcers per stomach were noted and severity of the ulcers were observed microscopically and scoring was done as per the method prescribed by S. K. Kulkarni [15]: 0 for normal coloured stomach, 0.5 for red coloration, 1 for spot ulcer, 1.5 for hemorrhagic streaks, 2 for ulcer between > 3 but < 5mm and 3 for ulcer > 5 mm. Mean ulcer score for each animal is expressed as ulcer index. The percentage protection was calculated.

2.4.2. Indomethacin induced ulcer

The albino rats of either sex weighing between 180-200 gm were divided into 4 groups of 6 animals each and fasted for 24 hrs with water *ad libitum* prior to experiment. The animals of group 1 were pretreated with vehicle and the animals of group 2 were treated with standard i.e. lansoprazole 8mg/kg. Similarly, the animals of group 3 and 4 were pre-treated with ethanol extract 200 mg/kg and 400mg/kg respectively. Indomethacin (30mg/kg p o) was administered to the animals of all groups, 60 minutes after the respective treatments. The animals were then sacrificed by cervical dislocation after 4 hrs. The stomach was taken out and cut open along the greater curvature of stomach. [17]. The ulcer index was scored as mentioned above by the method of S. K. Kulkarni [15] and percentage protection was also reported.

2.4.3. Pylorus-ligated (PL) induced rats

Albino rats of either sex weighing between 180-220 g were divided into 4 groups of 6 animals each and fasted for 18 hrs and care was taken to avoid coprophagy. Control vehicle (group-1) or standard drug (group-2) or extracts (group - 3 & 4) were administered 60 minutes prior to pyloric ligation under light ether anesthesia. The abdomen was opened and pyloric ligation was done without causing any damage to its blood supply. The animals were deprived of water during the post operative period. After 6 hrs, stomach was dissected out; contents were collected into tubes for estimation of biochemical parameters. The stomach was taken out and cut open along the greater curvature and ulcers were scored and % protection was reported as mentioned in the above explained models [15, 18].

Gastric Secretion - The gastric juice was collected 6 hrs after pylorus ligation and centrifuged for 5 minutes at 2000 rpm and the volume of supernatant was noted. The pH of the gastric juice was recorded by the pH meter. Then the contents were subjected to analysis for free and total acidity. Free acidity and total acidity were determined using 0.01N NaOH and Topfer's reagent containing phenolphthalein as indicator [15].

2.5. Statistical Analysis

Results were expressed as mean±SEM (n=6). Statistical analysis was performed with one way ANOVA followed by Turkey-Kramer multiple comparisons test. P value less than 0.05, was considered to be statistically significant ($p<0.05$).

3. RESULTS AND DISCUSSION

In the pylorus ligation induced ulcer model, HMEE at a dose of 200 and 400 mg/kg produced a reduction in the ulcer index, free acidity and total acidity significantly in comparison to the control group. The test extract reduced the gastric volume and raised the gastric pH significantly at a dose of 400 mg/kg. (Table no 1). In

the indomethacin induced ulcer model, the observations of positive control group indicated that indomethacin (30mg/kg) induced gastric ulcerations to the extent of 4.16±0.48 (ulcer index). Pretreatment with test extracts reduced the ulceration in a dose dependant manner. The extent of gastro-protective effect of the test extracts is 27.89% and 63.94% at 200mg/kg and 400mg/kg doses respectively, which is comparable to that of standard lansoprazole 8mg/kg. Similar results were obtained with ethanol induced ulcer model also. The test extract has shown gastro-protection in a dose dependant manner *i.e.* 56.64% and 66.24% protection at 200 and 400 mg/kg doses respectively. The test extracts at the doses mentioned above has shown significant protection even to that of standard lansoprazole (8mg/kg). The results are compiled in table 2.

Table 1: Effect of HMEE on Gastric Secretion following Pyloric Ligation induced Ulcer in Rats

Treatment	Dose	Volume(ml)	pH	Free Acidity(Eq/I)	Total Acidity(Eq/I)
Control	--	4.48± 0.27	1.99±0.23	30±1.826	97.74±9.178
Lansoprazole	**8 mg/kg BW**	1.77±0.2186***	5.97±0.41***	12.5±0.93***	27.33±2.028***
HMEE	**200 mg/kg BW**	3.96±0.46^{ns}	3.48±0.4681^{ns}	18.63±1.312**	56 ±4.252***
HMEE	**400 mg/kg BW**	3.38±0.36*	4.95±0.328***	15.39±1.242***	43.13±2.802***

*Values are the mean ± S.E.M. of six rats / treatment. Significance $^{ns}P>0.05$, *P <0.05, **P<0.001 and *** P<0.001 Vs. Control*

Table 2: Effect of HMEE on Indomethacin, Ethanol (1ml/200gm) and 6 hrs Pylorus ligation (PL) induced gastric ulcers in rats

Treatment	Dose	ULCER INDEX			% OF PROTECTION		
		Indomethacin	Ethanol	Pylorus Ligation	Indom-ethacin	Ethanol	Pylorus ligation
+ve Control	--	4.16±0.48	6.25±0.64	5.33±0.9189	-	-	-
Lansoprazole	**8mg/kg BW**	1.33±0.28***	1.42±0.40***	0.83±0.17***	68.03	77.28	84.43
HMEE	**200mg/kg BW**	3±0.22^{ns}	2.71± 0.38***	2.16±0.333***	27.89	56.64	59.47
HMEE	**400mg/kg BW**	1.5±0.45***	2.11±0.24***	1.58±0.4362***	63.94	66.24	70.36

*Values are the mean ± S.E.M. of six rats / treatment. Significance $^{ns}P>0.05$ and *** P<0.001 Vs. Control*

In the pyloric ligation induced ulcer model, there is increase level in acid-pepsin accumulation developed ulceration in rats due to pyloric obstruction and subsequent mucosal digestion [19]. After, administration of HMEE in pylorus ligated rats, it was observed that, gastric volume, ulcer index, free and total acidity were reduced and pH was increased. it is suggested that 70% HMEE can suppress gastric damage induced by aggressive factors.

Many previous studies have used ethanol as an ulcerogen. Alkofahi *et al* reported that ethanol (50 % v/v) induced ulceration in laboratory animals [20]. After ethanol administration, endothelin-1 is released which results in mucosal vasoconstriction. Under this condition, NO-induced vasodilation and its mucosal protective action is masked and gastric erosion is produced [21]. It has also been reported that leukotriene antagonist and 5-lipoxygenase inhibitors are capable of inhibiting alcohol induced gastric ulceration in rats [22]. HMEE significantly protected the gastric mucosa against ethanol challenge as shown by reduced values of ulcer index as compared to positive control group suggesting its potent gastroprotective effect. Similarly NSAID'S like indomethacin inhibits COX_1 thereby inhibits the prostaglandin synthesis, consequently lipooxygenase pathway is enhanced liberating leukotrienes and these leukotrienes are reported to have a role in ulcerogenesis. In addition there is some evidence that NSAIDs may induce ulcer by causing the back diffusion of H^+ ion in to mucosal cells [23]. Therefore the gastroprotective effect of the test extract may be due to its ability to inhibit the synthesis of prostaglandins/leukotrienes. In addition HMEE was significantly effective in protecting gastric mucosa against all the ulcerogenic models of the study. Hence, it may be inferred that HMEE affords effective protection to gastric mucosa against various insults may be by increasing gastric mucin content and increased the

pH and decreased the free and total acidity in rats, which in turn reduces the activity of pepsin and prevent mucolysis. This in turn protects the stomach from all the above mentioned challenges. Medical treatment of peptic ulcer is dependent on correcting the imbalance between the offensive and defensive factors. The test extracts acts on both the parameters of equation which govern the treatment of peptic ulcer and thus can be useful clinically. However further studies are needed to assess its safety profile before it is put into use clinically

4. CONCLUSION

The present study demonstrates the antiulcer activity of HMEE that could possibly due to the presence of phytochemical constituents *i.e.* flavonoids and saponins in the extract. There is room for further study to evaluate the active principles responsible for the antiulcer activity of the HMEE.

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