Research Article

International Journal of Pharmaceutical Biological and Chemical Sciences

ANTIULCER ACTIVITY OF *EUPHORBIA HIRTA* AGAINST EXPERIMENTALLY INDUCED ULCER IN RATS

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ABSTRACT:

Euphorbia hirta, an important medicinal herb, belongs to genus Euphorbia, family Euphorbiaceae, which has been presumed for its favorable medicinal effects. The present study was undertaken to evaluate the ethanolic extract of *Euphorbia hirta* aerial parts for its anti-ulcer activity by various gastric ulcer models. Oral administration of the *Euphorbia hirta* at 200 and 400 mg/kg significantly inhibited ulcer formation induced by various ulcer models like pylorus ligation, indomethacin, HCl/EtOH and restraint-stress in rats. In pylorus-ligated rats, pretreatment with the Euphorbia hirta extract had reduced gastric secretion. In HCl/EtOH induced ulcerated rats, gastric wall mucus was significantly preserved by the *Euphorbia hirta* pretreatment at doses of 200 and 400 mg/kg. The findings indicate that the ethanolic extract of *Euphorbia hirta* possesses gastroprotective potential which is related partly to preservation of gastric mucus secretion and anti secretary action.

KEYWORDS:

Euphorbia hirta, Pylorus ligation, antiulcer and gastroprotective

INTRODUCTION

Euphorbia is a genus of plants belonging to the family Euphorbiaceae. *Euphorbia hirta* is a very popular herb amongst practitioners of traditional herb medicine, widely used as a decoction or infusion to treat various ailments gastrointestinal disorders (including intestinal parasites, diarrhoea, peptic ulcers, heartburn, vomiting, amoebic dysentery), asthma, bronchitis, hay fever, laryngeal spasms, emphysema, coughs, colds, kidney stones, menstrual problems, sterility and venereal diseases. Moreover, the plant is also used to treat affections of the skin. The following gastroprotective potentials were isolated from the *Euphorbia hirta* Two flavonoids have been isolated from *Euphorbia hirta*, namely quercitrin and myricitrin[1,2]. Sterols were isolated from *Euphorbia hirta* and chemically characterized as cycloarternol, 24-methylene-cycloarternol, α-sitosterol, euphorbol hexacozonate, 1-hexacosanol, tinyaloxin, campesterol and stigmasterol[3,4]. *Euphorbia hirta* presents three hydrolysable tannins, namely, dimeric hydrolysable tannin, euphorbin E and the dimeric dehydroellagitannins, euphorbin A and euphorbin B[5]. The triterpenes α -amyrin, taraxerone (EH-1), taxerol as well as α -amyrin acetate have been identified from *Euphorbia hirta*[6,7].

Scientific evidence is available for most of the above mentioned ethnobotanical uses except peptic ulcer. The present study is, thus, aimed to evaluate anti-gastric ulcer effect of *Euphorbia hirta* using standard experimental models.

MATERIALS & METHODS

Plant Material

Aerial parts of *Euphorbia hirta* were collected from outskirts of Erode, Tamilnadu. Authentication has been done by Prof. V. S. Kumar, Scientists (F) and Head of the Office, Tamilnadu Agriculture University, Coimbatore (Tamilnadu). The voucher specimen (No.: BSI/ SRC/ 7/ 47/ 11- 12/ Tech. 221) has been deposited in the herbarium for future references.

Preparation of Extract

The aerial parts of *Euphorbia hirta* were washed with fresh water to remove adhering dirt and foreign particles. The plant was shade dried, crushed and grinded to get coarse powder. The coarse powder was then placed with 90% ethanolic solution in a round

bottomed flask. 500g of the coarse powder of the leaves of Euphorbia hirta in 1.0 liter of 90% ethanolic solution were macerated for 7 days. The mensturm was collected, concentrated by vacuum distillation and then air dried in an evaporating dish till constant weight was obtained.

Animals

Wistar albino rats of either sex weighing 150-200gm were used for this study. The animals were placed randomly and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±2^{o}C and relative humidity of 30-70%. A12:12 light: day cycle was followed. All the animals were allowed to free access to water and fed with standard commercial pelleted chaw (M/s.Hindustan Lever Ltd., Mumbai). All the experimental procedures and protocols used in this study were reviewed by (IAEC) Institutional Animal Ethics Committee (932/a/06/CPCSEA) of Sri Lakshminarayana Institute of Medical Sciences, Pondicherry and were in accordance with the guidelines of the IAEC.

Pharmacological Evaluation

Dose Schedule

The animals were divided into four groups each consisting of six rats. Group 1 represented Control group of animals received suspension of 0.1% CMC solution. Group 2 received Omeprazole (10 mg/kg). Groups 3 & 4, received *Euphorbia hirta extract*, in doses of 200and 400 mg/kg respectively. All the test drugs were administered orally by suspending in 0.1% CMC solution.

Pylorus ligation

All the test drugs were administered orally to 48 h fasted rats. One hour later, pylorus ligation as described by Shay et al[8]. Briefly, rats were lightly anesthetized by ether. The abdomen was opened and the pylorus was ligated. The abdomen was closed by suturing. The animals were sacrificed 5 h later by an overdose of ether. The stomach was removed and its content was subjected to measurement of volume and ulcer index.

Indomethacin Induced Gastric Ulcer

All the test drugs were administered orally to 48 h fasted rats 60 min prior to induction of gastric ulcers by indomethacin suspended in 0.5% carboxymethylcellulose at a single i.p. dose of 30 mg/kg [9]. After 5 h the rats were sacrificed and examined for gastric ulcers.

Restraint Water Immersion Stress-Induced Gastric Ulcer

All the test drugs were administered orally to 48 h fasted rats. Sixty minutes later, rats were restrained individually in stainless steel cages and immersed up to their xiphoid in a water bath maintained at 22±2 ◦C, according to the method of Takagi et al[10]. After 5 h of this exposure, the rats were sacrificed and examined for gastric ulcers.

HCl/EtOH Induced Gastric Ulcer

All the test drugs were administered orally to 48 h fasted rats 60 min prior to induction of gastric ulcers by 1.0 ml HCl/EtOH[11] (60 ml EtOH + 1.7 ml HCl + 38.3 ml H_2O) p.o. The animals were sacrificed and examined for gastric ulcers 60 min later and gastric wall mucus content.

Evaluation of the Gastric Ulcer

After each rat was sacrificed, the stomach was removed, opened along the greater curvature and the glandular portion of the stomach was examined. The length in mm of each lesion was measured under a dissecting microscope and the sum of the length of all lesions was designated as the ulcer index.

Determination of Gastric Wall Mucus Content

Gastric wall mucus was determined by the Alcian blue method[12]. The stomach was excised from the sacrificed animals and opened along the lesser curvature, weighed and immersed in 0.1% w/v Alcian blue solution for 2 h. The excessive dye was then removed by two successive rinses in 0.25M sucrose solution. Dye complexed with gastric wall mucus was extracted with 0.5M $MgCl_2$ for 2 h. The blue extract was then shaken vigorously with an equal volume of diethyl ether and the resulting emulsion was centrifuged. The optical density of Alcian blue in the aqueous layer was read against a buffer blank at 580 nm using a spectrophotometer. The quantity of Alcian blue extract per gram wet stomach was then calculated from a standard curve.

Statistical Analysis

The values were expressed as mean ± SEM. The statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Dunnet's 't' – test. P values <0.05 were considered significant.

RESULTS

The antiulcer effect of *Euphorbia hirta* at 200 & 400mg/kg dose levels were studied in pylorus ligation, indomethacin, cold & restraint and HCl/Ethanol induced ulcers. The *Euphorbia hirta*

showed significant protective effect at both the doses on all models. The effect of *Euphorbia hirta* on ulcer index and % protection against above mentioned ulcer models were shown in table 1 and table 2 respectively. In pyloric ligation model, *Euphorbia hirta* at doses of 200 and 400 mg /kg inhibited ulcer formation significantly (50.46% and 87.43% respectively). Omeprazole, the standard antiulcer agent, significantly (94.78%) inhibited the ulceration induced by pyloric ligation.

Table 1- Shows the effect of *Euphorbia hirta* extract on ulcer index of various ulcer models in rats

Drug Treatment	Ulcer Index			
	Pylorus ligation	Indomethacin	Cold and restraint	Ethanol/ HCL
Control 0.1 % CMC (1 ml / kg)	75.15 ± 5.12	19.66 ± 1.85	15.67 ± 1.44	72.53 ± 4.61
Omeprazole (10mg/kg)	3.92 ± 0.45***	3.17 ± 0.62***	6.12 ± 0.60***	2.67 ± 0.57***
Euphorbia hirta (200mg/kg)	37.25 ± 3.66***	7.44 ± 0.64***	13.25 ± 1.33***	7.45 ± 0.71***
Euphorbia hirta (400mg/kg)	9.45 ± 0.63***	6.57 ± 036***	9.63 ± 0.92***	4.99 ± 0.36***

Values are presented as mean ± SEM (n = 6)
*P<0.05, **P<0.01 and ***P<0.001 Vs control

Table 2- Shows the Percentage protection of ulcer by *Euphorbia hirta* extract on various ulcer models in rats

Drug Treatment	% Ulcer Protection			
	Pylorus ligation	Indomethacin	Cold and restraint	Ethanol/ HCL
Control 0.1 % CMC (1 ml / kg)	-	-	-	-
Omeprazole (10mg/kg)	94.78	83.93	60.95	96.32
Euphorbia hirta (200mg/kg)	50.46	62.27	15.46	89.72
Euphorbia hirta (400mg/kg)	87.43	66.69	61.43	93.11

Administration of indomethacin resulted in the production of gastric lesions mainly in the glandular portion of the stomach. The rats treated with *Euphorbia hirta* significantly (p<0.001) decreased the intensity of gastric mucosal damage induced by indomethacin. The % protection of gastric lesion was more (66.69%) in the groups of animals received *Euphorbia hirta* 400 mg/kg when compare to *Euphorbia hirta* at 200 mg/kg (62.27). Animals subjected to cold & restraint for 3 h showed the presence of ulcer in glandular portion of stomach. Treatment with *Euphorbia hirta* 400 mg/kg (61.43%) produced a marked increase in ulcer protection when compared to 200mg/kg (15.46%). In ethanol/HCL model the *Euphorbia hirta* (200 and 400mg/kg) showed a significant reduction in ulcers index at both the doses by 89.72% and 93.11% respectively.

The effect of *Euphorbia hirta* extract on gastric wall mucus content in rats was estimated in HCl/Ethanol induced ulcers and the results were shown on table 3. *Euphorbia hirta* at doses of 200 and 400 mg/kg significantly restored the mucus content. The mucus content in ulcerated group was 325.55±18.45 as compared to *Euphorbia hirta* 200 and 400 mg/kg treated (415.36±21.44 and 425.15±29.64 respectively) groups.

Table 3- Shows the effect of *Euphorbia hirta* extract on gastric wall mucus content in rats

Drug Treatment	Gastric wall mucus (µg Alcian blue/g wet stomach)
Control 0.1 % CMC (1 ml / kg)	325.55 ± 18.45
Omeprazole (10mg/kg)	398.25 ± 24.15*
Euphorbia hirta (200mg/kg)	415.36 ± 21.44**
Euphorbia hirta (400mg/kg)	425.15 ± 29.64**

Values are presented as mean ± SEM (n = 6)
*P<0.05, **P<0.01 and ***P<0.001 Vs control

DISCUSSION

According to the experimental models used in this study, NSAIDs like indomethacin induce ulcer formation by depleting cytoprotective PGs. PGE2 and PGI2 of gastric and duodenal mucosa are responsible for mucus production and maintaining cellular integrity of the gastric mucosa[13]. In the HCl/EtOH induced gastric ulceration model, HCl causes severe damage to gastric mucosa[14] whereas ethanol produces necrotic lesions by direct necrotizing action which in turn reduces defensive factors, the secretion of bicarbonate and production of mucus[15]. The water immersion stress-induced ulcers are caused by an increase in gastric acid secretion[16] and decreases in mucosal microcirculation[17] and mucus content[18]. The finding that the *Euphorbia hirta* reduced the ulcer index which is due to the decrease in gastric volume in pylorus-ligated rats suggests that anti-secretory action may be responsible for anti-gastric effect of the *Euphorbia hirta*. The gastric wall mucus is thought to play an important role as a defensive factor against gastrointestinal damage[19]. The determined gastric wall mucus was used as an indicator for gastric mucus secretion[20]. The finding that pretreatment with the H hirta at doses of 200 and 400mg/kg significantly increased gastric mucus content in HCl/EtOH ulcerated rats suggests that the gastroprotective effect of the *Euphorbia hirta* is mediated partly by preservation of gastric mucus secretion.

CONCLUSION

In conclusion, this study provides evidence that the ethanolic extract of *Euphorbia hirta* possesses gastroprotective activity which may be due to the preservation of gastric mucus secretion and inhibition of gastric acid secretion.

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