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## Research Article

### EVALUATION OF ANTI ULCER PROPERTY OF CARDIOSPERMUM HALICACABUM LINN. LEAF EXTRACT

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#### ABSTRACT

The present study was carried out to evaluate the anti ulcer potential of ethanolic leaf extract of *Cardiospermum halicacabum* Linn. against Indomethacin induced gastric ulcer model in male Wister rats. The ethanolic extract of *C. halicacabum* (EECH) was administered at a dose of 200 and 400mg/kg orally 30 min. prior to ulcer induction. Omeprazole (10mg/kg/day p.o) was used as a standard. The anti ulcer activity was assessed by determining the ulcer index in the test group along with the Standard drug treated group. EECH registered a significant dose dependent decrease in the extent of gastric mucosal damage in the induced ulcer models. Presence of bioactive compounds like flavonoids, tannins, phenols, saponins and caffeic acid may be responsible for the anti ulcerogenic activity of the present study plant.

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#### INTRODUCTION

Plants are untapped reservoir of various phytochemicals awaiting intensive exploitation for their biological properties. Regular survey of specific medicinal plants in different parts of India is very much needed to emphasize the importance of medicinal plants in their populations in various geographical regions to find out their utility to the Indian Pharmaceutical Standards (Rao *et al.*, 2000). Many of the plant species that proved as medicinal herbs have been scientifically evaluated for their possible medical applications (Saxena *et al.*, 2014 ; Xiao, 2015).

Peptic ulcer disease is a common health problem worldwide now-a-days. It is a break off in the continuity of the mucosa of stomach or duodenum (Verma, 2010) to pepsin and gastric acid. It is caused by an imbalance between the protective (mucus, bicarbonate, NO and PG's) and the aggressive mechanism of the mucosa or association of several endogenous factors like hydrochloric acid and pepsin and exogenous factors such as tobacco, alcohol, NSAIDS and *Helicobacter pylori* infection (Rakesh Pahwa *et al.*, 2010).

A number of drugs are available for the treatment of peptic ulcer but its clinical evaluation shows the incidence of relapses, side effects and drug interaction. This emphasizes the development of new anti ulcer drug and search for novel molecules. The treatment of peptic ulcers with novel molecule from plant products using medicinal plants received more attention (Vimala and Gricilda Shoba, 2014; Ahmad *et al.*, 2015).

*Cardiospermum halicacabum* Linn. belongs to the family Sapindaceae is an annual climber. It is traditionally used in the treatment of chronic bronchitis, stiffness of limbs and snake bites (Chopra, 1992), diuretic, laxative (Kirtikar and Basu, 2001), anti malarial (Wakko *et al.*, 2005) and hepato protective (Rupeshkumar *et al.*, 2012). Hence the present work has been carried to prove the folk claim of leaves of *C. halicacabum* against anti ulcer activity.

#### MATERIALS AND METHODS

The leaves of *Cardiospermum halicacabum* L. were collected from Thennilai, near Karur District in Tamil Nadu and was identified and authenticated by Botanical Survey of India,

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Coimbatore whose voucher specimen no. is BSI /SRC / 5 / 23 / 2013-14 / Tech / 202.

### Preparation of Plant Extract

The leaves of *C. halicacabum* were shade dried at room temperature. The dried material was then homogenized to obtain coarse powder and stored in air tight bottles for further analysis.

Coarse powder from the shade dried plant material was exhaustively extracted with ethanol to yield a dark greenish semisolid residue. The dried extract was dissolved in distilled water right before use.

### Animals

The experiment was carried out using Male Wister albino rats weighing about 150-175 g. The animals were procured from the small animals breeding station, Mannuthy, Kerala, India. Animals were housed under standard conditions of temperature ( $23 \pm 1^\circ \text{C}$ ), relative humidity ( $55 \pm 1^\circ \text{C}$ ), 12 h/ 12h/ light/dark cycle and fed with standard pellet diet (Pranav Agro Industries Ltd., Sangli, India) and water *ad libitum* and were allowed to acclimatize for seven days to laboratory condition before the experiment. All the experimental procedures and protocols used in this study were received by the Institutional Animal Ethics Committee (Reg. No. ML-EA-CPCSEA / 01-2013 /05) and were in accordance with the guidelines of CPCSEA

### Acute toxicity test for LD 50 determination (Sathya et al., 2011)

Acute toxicity studies were carried out to study the toxic effects of the drug. About thirty six Male Wister Albino rats weighing about 150-175 g distributed into six groups comprising of a control group and five treated groups with 6 animals per group were used. The treated groups were given varying doses of 50,100,500,1000 and 2000 mg / kg body weight of ethanolic plant extract. Control animals received 5% Acacia as control. After the administration of plant extract, the animals were observed for 42 h to detect any changes in autonomic or behavioural responses viz. irritability, corneal reflex, hyperactivity, sedation, urination, salivation and death. All the animals were observed twice daily for any mortality during the experimental period.

### Indomethacin (IND) induced ulcers

The experiment was performed according to the method of Djahanguiri (1969). Four groups of Male Wister rats (n = 6) were fasted over night prior to the start of experiment, and water *ad libitum*. The first group received distilled water, while the second group treated with omeprazole (10 mg / kg /day,p.o). Whereas third and fourth groups were administered with ethanolic extract of *C. halicacabum* , 200 and 400 mg / kg /day p.o, respectively. On day third after 30 min. of omeprazole and plant extract treatments, indomethacin (50 mg / kg) suspended in 5% carboxy methyl cellulose was given as a single oral dose to group 2-4 to induce gastric ulcers. After 5 h, the animals were sacrificed with over dose of diethyl ether and each stomach was examined for ulcer index.

### Experimental design

Group I : Control which received distilled water orally  
Group II : Omeprazole (10mg/kg/day p.o)

GroupIII : Served as test sample which received ethanolic leaf extract of 200mg/ kg p.o of *C.halicacabum*

GroupIV : Served as test sample which received ethanolic leaf extract of 400mg/kg p.o of *C. halicacabum*

### Ulcer Index

The stomachs were removed and opened along the greater curvature, washed gently in normal saline and the mean ulcer index was calculated. The number of ulcer lesions was counted using a magnifying glass and the diameter of the ulcer was measured using a vernier caliper. Ulcer index was determined by following the scoring method of Suzuki et al. (1998).

Score 1: maximal diameter of 1 mm  
Score 2: maximal diameter of 1-2 mm  
Score 3: maximal diameter of 2-3 mm  
Score 4: maximal diameter of 3-4 mm  
Score 5: maximal diameter of 4-5 mm  
Score10: maximal diameter of 5 mm and above  
Score25: a perforated ulcer

The sum of the length (mm) of all lesions for each stomach was used as the ulcer index (UI) and the protection percentage was calculated from the following formula

$$\text{Percentage Protection} = \frac{\text{UI control} - \text{UI treated}}{\text{UI control}} \times 100$$

### Statistical analysis (Steel and Torrie, 1960)

The data presented are means  $\pm$  SD of 6 rats in each group. The results were analyzed using one-way analysis of variance (ANOVA) and the group means were compared by Duncan's Multiple Range Test (DMRT) using Statistical Program for Social Sciences (SPSS version 16.0) software for windows. The findings were considered statistically significant at  $p < 0.05$ .

## RESULTS AND DISCUSSION

Phytochemical analysis of ethanolic extract of *C. halicacabum* showed the presence of carbohydrates, flavonoids, phenols, saponins, steroids, tannins, terpenoids, rutin, gallic acid, quercitrin and caffeic acid ( already published data )

### Acute toxicity studies

Acute toxicity studies were carried out on rats for EECH at doses of 50, 100, 500, 1000 and 2000 mg/kg body weight. The acute toxicity of extract showed no mortality and morbidity even under high dose levels (2000mg/k b.w.) indicating high margin of safety of the plant extract.

### Indomethacin induced gastric ulcer model

Gastric lesions were induced in rats by oral administration of Indomethacin (50mg/kg), Oral administration of EECH registered a significant dose dependent decrease in the extent of gastric mucosal damage in Indomethacin induced ulcer models (Table 1, Plate I ). Ulcer score reduced at both doses but at a dose level of 400mg /kg b.w. showed high protection percentage compared to 200mg /kg b.w. Hence though the two doses of extracts have anti ulcer activity, EECH at 400 mg/ kg b.w. seems to be more potent. Percentage protection (60.94%) by the usage of high dose (400mg/kg b.w.) seems to be closer

to the standard omeprazole. It is in consonance with the earlier studies in *Aloe vera* (Borra *et al.*, 2011); *Mangifera indica* (Neelima *et al.*, 2012) and *Shorea robusta* (Sathishkumar *et al.*, 2013).

**Table 1** Effect of ethanolic extract of *Cardiospermum halicacabum* leaves on ulcer index of indomethacin induced gastric ulcer in rats<sup>a</sup>

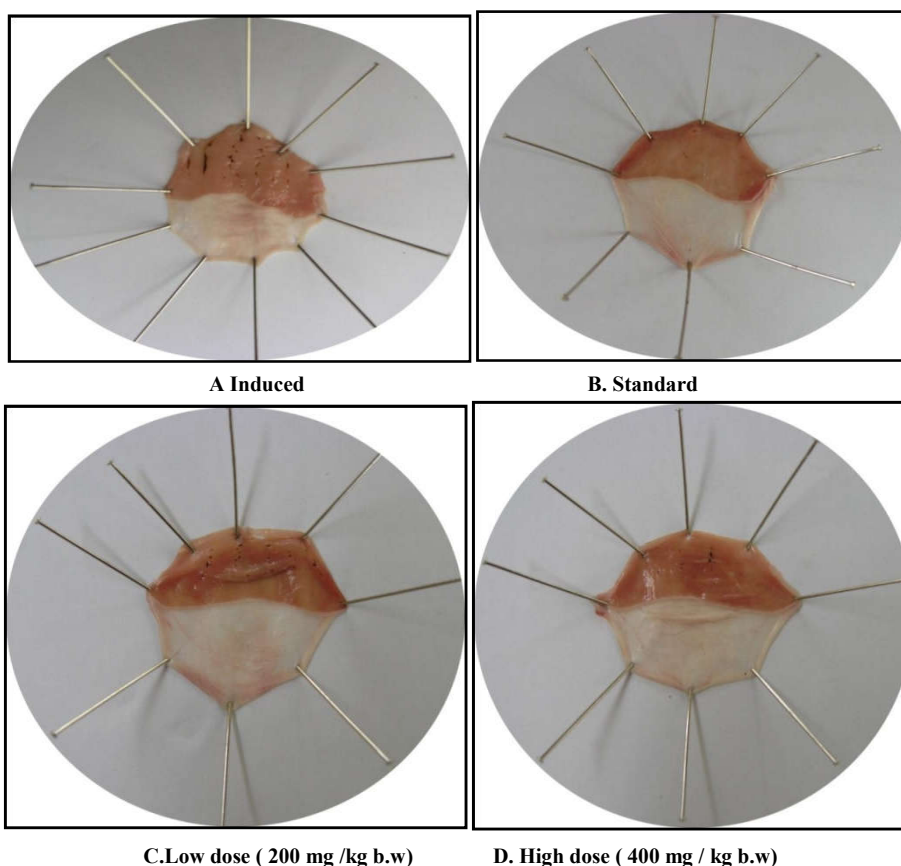
Group	<i>C. halicacabum</i>	
	Ulcer Score	Percentage protection (%)
Induced	35.15 <sup>a</sup> ± 0.99	-
Standard	10.33 <sup>d</sup> ± 0.69	70.61
Low dose (200mg kg <sup>-1</sup> b.w)	22.19 <sup>b</sup> ± 1.05	36.87
High dose (400mg kg <sup>-1</sup> b.w)	13.73 <sup>c</sup> ± 0.76	60.94

<sup>a</sup> Values are expressed as mean ±SD for 6 animals (n=6) significant at p<0.05 level. One way ANOVA followed by Duncan's Multiple Range Test.

Flavonoids showed cytoprotective action by increasing mucosal content of prostaglandins and mucous in gastric mucosa (Mohammed *et al.*, 2008; Patil *et al.*, 2008; Kaur *et al.*, 2012). Free radical scavenging ability of flavonoids have also been reported to protect the gastro intestinal tract from ulcerative and erosion lesion (Gupta *et al.*, 2005).

Anti ulcerogenic activity of saponin has been confirmed in earlier investigations (Uma *et al.*, 2009; Borra *et al.*, 2011; Kumar *et al.*, 2013). Saponins likely to be inhibit gastric mucosal injury by scavenging the IND-or stress generated oxygen metabolites (Shetty *et al.*, 2008).

Quercetin and caffeic acid have been reported to prevent gastric mucosal lesions in various experimental models (Di Carlo *et al.*, 1999; Panda and Sonkamble, 2012) by increasing the amount of neutral glyco proteins.



The phytochemical constituents such as tannins, phenols, flavonoids, terpenoids and saponins have been reported to be the possible potent gastro protective agents in previous studies. Anti ulcer efficacy of flavonoids, tannins and triterpenes have been extensively confirmed by Borelli and Izzo (2000). It may be suggested that these active compounds would be able to stimulate mucus, bicarbonate and the prostaglandin secretion and counter act with the deteriorating effects of reactive oxidants in gastro intestinal lumen (Sakat and Juvekar, 2009).

Tannin may act as a potent gastro protective agent due to their mucosal membranes protein precipitation and vasoconstriction effects. Astringent action of tannin may also help in precipitating micro proteins on the ulcer sites and protects the underlying mucosa from toxins and other irritants (Duke *et al.*, 2003; Berenguer *et al.*, 2005; Kumar *et al.*, 2013).

### Summary

In conclusion the leaves of *C. halicacabum* possess significant anti ulcer activity may probably due to the presence of interesting bioactive compounds like flavonoids, tannins, phenols, saponins and caffeic acid. The obtained results also suggested that *C. halicacabum* may serve as a better alternate potent anti ulcer agent after conducting human trial experiments under clinical conditions. The integration of phytomedicine in to the health system should be developed in such a way to bring compatibility between the traditional and modern system of health care with minimum threat to each other.

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