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Evaluation of anti-ulcer activity of ethanolic leaf extract of *Acalypha Indica* Linn

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Abstract
The objective of present study is to evaluate the antiulcer activity of ethanolic leaf extract of *Acalypha indica*. The cause of ulceration in patients is mainly due to hyper secretion of gastric acid and pepsin. *Acalypha indica* plant extract some of the most attractive sources of new drugs and have been shown to produce promising results in the treatment of gastric ulcers. The anti-ulcer activity of ethanolic extract of *Acalypha indica* was investigated by pylorus ligation and indomethacin induced gastric ulcer in rats.

Keywords: Pylorus ligation, indomethacin, anti-ulcer activity, *Acalypha indica,* ethanolic extract

Introduction
Peptic ulcer is one of the most prevalent diseases around the world affecting four million people each year. Peptic ulcer is the term which refers to acid peptic injury of the digestive tract, and it results in mucosal break reaching the sub mucosa [1]. The disease involves an imbalance between offensive and defensive factors such as pepsin, acid and *Helico bacter pylori* and bicarbonates, prostaglandins, mucin, nitric oxide and growth factors, respectively [2]. It has been also found that there is a chronic remitting course of peptic ulcer disease with imperfect correlation between symptoms and the presence of ulcer [3]. *Helicobacter pylori* infection is a very common cause of primary peptic ulcers. It is associated with 70% of gastric ulcers and 90% of duodenal ulcers [4]. Other risk factors responsible to produce peptic ulcer disease are alcohol consumption, cocaine, tobacco and amphetamine use, chronic administration of non-steroidal anti-inflammatory drugs (NSAIDs), Fasting, Zollinger-Ellison Syndrome, and cancer treatment with angiogenesis inhibitors [5, 6].
Peptic ulcer treatment involves using a number of chemically produced drugs with aim to reduce the rate of stomach acid secretion, protection of the mucosa that line the stomach and upper portion of the small intestine or to eliminate *H. Pylori* infestation [7]. The existing drugs cause several adverse effects, conversely, indigenous herbal drugs are devoid of side effects which might better treat peptic ulcers [8]. Medicinal plants possess numerous active phyto constituents that are responsible for several biological activities. *Acalypha indica* is very important medicinal plant, to possess anti-ulcer activity [9].

Materials and Methods
The experiment was carried out by male wister albino rats weighing (150-175 g) and were procured from the small animals breeding station, Mannuthy, Kerala, India. The animals were housed under standard conditions of temperature (23±1 ºC), relative humidity (55±1 ºC), 12h/12h light/dark cycle and fed with standard pellet diet (Pranav Agro Industries Ltd., Sangli, India) and water *ad libitum*. Animal described as tested were deprived of food for atleast 18h but allowed free access to water. All the experimental procedures and protocols used in the study were received by the Institutional Animal Ethics Committee (Reg no: 688/Z/C-CPCSEA) and were in accordance with the guidelines of the CPCSEA.

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

Indomethacin (IND) induced ulcers

The experiment was performed according to the method of Djahanguiri (1969). Four groups of male Wister rats (n=4) were fasted overnight prior to the start of the experiment and water *ad libitum.* The first group received distilled water, which the second group was treated with omeprazole (10 mg kg^{-1} day^{-1} P.O.). Whereas third to fourth groups were administrated with the ethanolic extract of present study plants (200 and 400 mg kg^{-1} day^{-1} P.O. respectively). On day third after 30 min of omeprazole and plant extract treatments, indomethacin (50 mg kg^{-1}) suspended in 0.5% carboxymethyl, cellulose was given as a single oral dose to groups 2-4 to induce gastric ulcers. After 5h, the animals were sacrificed with over dose of diethyl ether and each stomach was examined for ulcer index [10].

Experimental Design

- **Group – I:** Control which received distilled water orally.
- **Group – II:** Omeprezole (10 mg kg^{-1} day^{-1} P.O.).
- **Group – III:** Served as test sample which received ethanolic leaf extract of 200 mg kg^{-1} P.O of *Acalypha indica.*
- **Group – IV:** Served as test sample which received ethanolic leaf extract of 400 mg kg^{-1} P.O of *Acalypha indica*.

Ulcer Index: The stomach 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 were counted using a magnifying glass and the diameter of the ulcer was measured using a vernier calliper. Ulcer index was determined by following the scoring method of Suzuki *et al*., 1998 [11].

- **Score 1:** Maximal diameter of 1mm
- **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
- **Score 10:** Maximal diameter of 5 mm and above
- **Score 25:** 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:

$$\frac{\text{Percentage}}{\text{Protection}} = \frac{\text{UI Control} - \text{UI Treated}}{\text{UI Control}} \times 100$$

Statistical Analysis

The data presented here are means ± SD of 6 rats in each group. The results were analysed 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 significantly at $p < 0.05$ [12].

Results and Discussion

The ethanolic extract of *Acalypha indica* (Table 1 and Fig 1) found to possess significant anti ulcero genic activity with a dose dependent manner. They have a gastric anti secretory, acid neutralizing effect and anti-oxidative effect. The gastro protective effect of *Acalypha indica* may probably due to presence of bioactive compounds like Flavonoids, Saponin, Alkaloids, Glycosides. The results suggested that the plant of *Acalypha indica* may serve as an alternate potent anti-ulcer agent.

Gastric lesion were induced in rats by oral administration of Indomethacin. Oral administration of ethanolic leaf extract of *Acalypha indica* registered a significant dose dependent decrease in the extent of gastric mucosal damage in Indomethacin induced ulcer models. Indomethacin induced depletion of gastric wall mucous formation has been significantly reduced by ethanolic leaf extract of *Acalypha indica* at a dose levels of 200 mg kg^{-1} b.w and 400 mg kg^{-1} b.w showing protection percentage as 48.13 and 67.72, respectively (Table-1).

Table 1: Effect of ethanolic extract of *acalypha indica* leaves on ulcer index of indomethacin induced gastric ulcer in rats[a]

Group	Ulcer Score	Percentage Protection (%)
Induced	36.17^{a} ±0.98	-
Standard	11.37^{d} ±0.72	72.44
Low dose (200 mg kg^{-1} b.w)	19.87^{b} ±0.36	48.13
High dose (400 mg kg^{-1} b.w)	13.95^{c} ±0.38	67.72

Values are expressed as mean ± SD for 4 animals (n=4) significant at $p < 0.05$ level.

One way ANOVA Followed by DUNCAN'S Multiple Range Test.

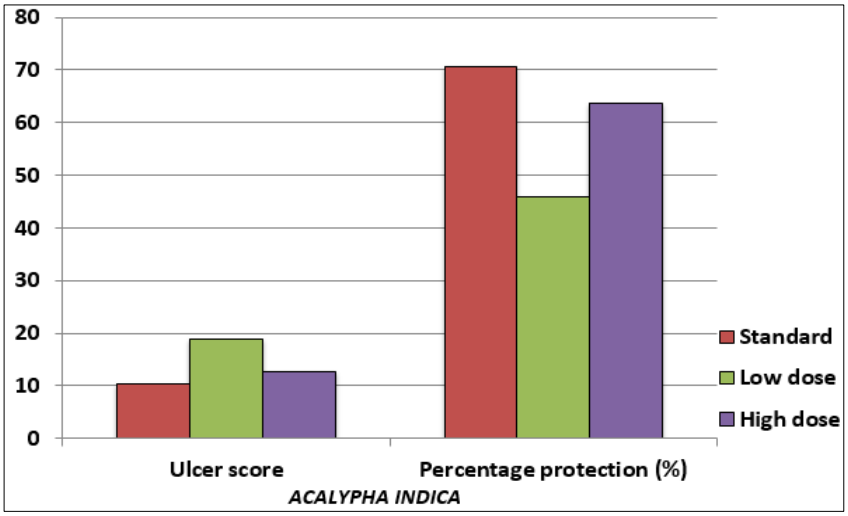

Fig 1: Percentage protection of ethanolic extract of *acalypha indica* leaves on ulcer index of indomethac in induced gastric ulcer in rats

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