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# EVALUATION OF ANTIULCER ACTIVITY OF *CALOTROPIS GIGANTEA* R.Br LEAVES

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## ABSTRACT

The antiulcer activity of ethanolic extract of *Calotropis gigantea* R.Br leaves (EECG) was investigated in pylorus ligation and ethanol induced ulcer models in experimental rats. In both models the common parameter determined was ulcer index. Ethanolic extract of *Calotropis gigantea* at a dose of 150 and 300mg/kg produced significant inhibition of the gastric lesions induced by pylorus ligation induced ulcer and ethanol induced gastric ulcer. The extract (150mg/kg and 300mg/kg) showed significant ($p<0.05$) reduction in gastric volume, free acidity and ulcer index as compared to control. This present study indicates that EECG have potential anti-ulcer activity in both models. These results may further suggest that the extract was found to possess antiulcerogenic as well as ulcer healing properties, which might be due to its antisecretory activity.

**INTRODUCTION:** Peptic ulcer is one of the most common gastrointestinal diseases [1]. The exact causes of peptic ulcer disease are not known but it may result from an imbalance between acid-pepsin secretions and mucosal defense factors [2]. Peptic ulcer disease occurs mainly due to consumption of NSAIDS, infection by H.pylori, stress or due to pathological condition such as Zollinger-Ellison Syndrome [3].

*Calotropis gigantea* is commonly known as 'madar' in Hindi, belonging to the family Asclepiadaceae, is a milky shrub up to 1-3m in height found through out India [4, 5]. On phytochemical investigation researcher claimed that leaves of *Calotropis gigantea* found to contain a resin-mudarine, glycosides viz., calotropin, uscharin and calotoxin [6].

The leaf has been found to show anti microbial activity [7], nematicidal activity [8], wound healing activity [9], anti-inflammatory activity [10], anti-diarrheal activity [11], hepatoprotective activity [12], antipyretic activity [13], anti-arthritic activity [14], hypoglycemic activity [15], anti-Candida activity [16].

So for no systematic study has been reported for antiulcer properties of *Calotropis gigantea* leaf extracts. In the present study effort has been made to establish the scientific validity to the anti ulcer property of *Calotropis gigantea* leaves extracts using pyloric ligation and ethanol induced ulceration models in albino rats.

## MATERIALS AND METHODS:

**Plant materials:** The leaves of *Calotropis gigantea* were collected in and around the kundrathur hill, Anakaputur, Chennai, Tamil Nadu in the month of November, 2010. The specimen was identified and authenticated by Prof. Dr. P. Jayaraman, Director, Plant Anatomy Research Center (PARC), Tambaram, Chennai.

The specimen was deposited to herbarium of SRM College of Pharmacy. The voucher specimen no was (PARC/2010/598). After authentication, fresh leaves collected in bulk from plants, washed, shade dried and then milled to a coarse powder by a mechanical grinder,

**Preparation of extract:** The powders of dried leaves were packed in to soxhlet column and extract with ethanol. The extract was filter through a Whatmann filter paper no.1 and concentrated under reduced pressure (yield of extract was 9.40% with respect to dry material). Just prior to use, the substance was dissolved in physiological saline solution.

**Animals:** The study was conducted on male Wister rats (175-200gm) housed in polypropylene cages under standard conditions of temperature (22 ± 2°C) relative humidity (60 ± 5%) and light (12h light/dark cycle) were used. They were fed with standard diet and water. The food was withdrawn 18 hours before the experiment but allowed free access of water. All animal experiments were carried out in accordance with the guidelines of CPCSEA.

**Acute Oral Toxicity Studies:** Acute toxicity was carried out according to Organization of Economic Co-Operation and Development (OECD) guidelines[17], No mortality was observed and all the test doses were found to be safe.

**Pyloric ligation in rats:** The animals were divided into 5 groups, each consisting of six rats. Control group received distilled water only. Second group of rats are pyloric ligated. Third and fourth groups received EECG in a dose of 150 and 300 mg/kg. The fifth group of animals received Omeprazole in the dose of 20mg/kg as a reference drug for ulcer protective studies. After 45 min of the treatment, pyloric ligation was done by ligating the pyloric end of stomach of rats of respective groups under ether anesthesia at a dose of 35mg/kg of body weight.

Ligation was done without causing any damage to the blood supply of the stomach. Animals were allowed to recover and stabilize in individual cages and were deprived of water during post-operative period. Rats were sacrificed after 4hr of surgery and ulcer scoring was done.

Gastric juice was collected and gastric secretion studies were performed according to the standard procedure [18].

**Ethanol induced Ulcer Model:** The ulcer was induced by administering absolute ethanol (1ml/200g). All the animals were fasted for 36 hours and then ethanol was administered to induce ulcer. The animals were divided into five groups, each consisting of six rats. The control group received distilled water, second group received ethanol. Third and fourth groups received EECG in a dose of 150 and 300 mg/kg. The fifth group of animals received Omeprazole in the dose of 20 mg/kg as a reference drug. They were kept in specially constructed cages to prevent coprophagia during and after the experiment. The animals were anaesthetized 1 hr later with anaesthetic ether and stomach was incised along the greater curvature and ulceration was scored. A score for the ulcer was studied to pyloric ligation induced ulcer model[19].

**Scoring of ulcers:**

| | | |
|---|---|---|
| Normal stomach | : | -0 |
| Red coloration | : | -0.5 |
| Spot ulcer | : | -1 |
| Hemorrhagic streak | : | -1.5 |
| Ulcers (< 2mm) | : | -2 |
| Ulcers (>2 < 4 mm) perforation | : | -3 |
| Ulcers (< 4mm) | : | -4 |

Mean ulcer score for each animal was expressed as ulcer index. The percentage of ulcer protection was determined by;

$$\% \text{ of ulcer protection} = \frac{\text{Control mean ulcer index} - \text{Test mean ulcer index}}{\text{Control mean ulcer index}} \times 100$$

**Determination of free acidity:**

$$\text{Acidity} = \frac{\text{Volume of sodium hydroxide} \times \text{Normality} \times 100\text{mEq/L/100g}}{0.1}$$

**Statistical analysis:** The values are represented as mean ± S.E.M, and Statistical significance between treated and control groups was analyzed using of one way ANOVA, followed by Dennett's test where $P<0.05$ was considered statically significant.

**RESULTS:**

**Pyloric Ligation induced Gastric Ulcer:** In pyloric ligation induced ulcer model, oral administration of EECG in two different doses showed significant reduction in ulcer index, gastric volume, free acidity, total acidity compared to the central group. EECG exhibited a protection index of 68.7% and 81.2% at the dose of 150 and 300 mg/kg respectively, where as Omeprazole as reference standard exhibited a protection index of 85.2% (**Table 1**).

**Ethanol-induced Gastric Ulcer:** In control animal, oral administration of absolute ethanol produced characteristic lesions in the glandular portion of rat stomach which appeared as elongated bands of thick, blackish red lesions. EECG has shown significant protection index of 67.7% and 71.2% with the dose of 150 and 300 mg/kg respectively whereas Omeprazole as reference standard showed protection index of 79.6% (**Table 2**).

**TABLE 1: EFFECT OF EECG ON VARIOUS PARAMETERS IN PYLORIC LIGATION INDUCED GASTRIC ULCERS**

| Group | Treatment | Ulcer index | Free acidity meq/ltr | $P^H$ of gastric juice | Gastric juice | Total acidity meq/ltr | Protection (%) |
|---|---|---|---|---|---|---|---|
| I | Normal (distilled water) | --- | 41.3 ± 0.3 | 5.41 ± 0.3 | 3.8 ± 0.4 | 62.3 ± 0.2 | --- |
| II | Control (pyloric ligation) | 14.2 ± 1.2 | 95.6 ± 1.4 | 2.51 ± 0.2 | 8.2 ± 0.2 | 112.5 ± 0.2 | --- |
| III | EECG (150mg/kg) | 4.5 ± 0.5 | 43.7 ± 0.3 | 4.87 ± 0.2* | 5.3 ±1.2 | 75.3 ± 0.4 | 68.7 % |
| IV | EECG (300mg/kg) | 2.8 ± 0.4* | 39.8 ± 0.2* | 5.51 ± 0.4* | 4.2±0.4* | 61.7 ± 0.6* | 81.2% |
| V | Omeprazole (20mg/kg) | 2.2 ± 0.5* | 37.4 ± 0.2* | 5.71 ± 0.4* | 3.9 ± 0.2* | 60.1 ± 1.4* | 85.2% |

**TABLE 2: EFFECT OF EECG ON VARIOUS PARAMETERS IN ETHANOL INDUCED GASTRIC ULCERS**

| Group | Treatment | Ulcer index | $P^H$ of gastric juice | Protection (%) |
|---|---|---|---|---|
| I | Normal (distilled water) | --- | 5.42 ± 0.3 | --- |
| II | Control (pyloric ligation) | 12.3 ± 0.2 | 2,83 ± 0.6 | --- |
| III | EECG (150mg/kg) | 4,3 ± 0.5 | 3.68 ± 0.6 | 67.7% |
| IV | EECG (300mg/kg) | 3.6 ± 0.4* | 4.86 ± 0.7* | 71.2% |
| V | Omeprazole (20mg/kg) | 2.7 ± 0.4* | 5.62 ± 0.7* | 79.6% |

Values are expressed as mean ± SEM of observations, Statistical comparisons as follows: Significant *P <0.005 compared to control group

**DISCUSSION:** The etiology of peptic ulcer is unknown in most of the cases, it is generally accepted that gastric ulcer results from an imbalance between aggressive factors and the maintenance of the mucosal integrity through the endogenous defense mechanism [20]. Different therapeutic agents are used to inhibit the gastric acid secretion or to boost the mucosal defense mechanisms by increasing mucosal production, stabilizing the surface epithelial cells or interfering with the prostaglandin synthesis [21]. The prostaglandins can provide gastric cytoprotection in rats against strong necrotizing irritants without reducing gastric acid secretion [22].

The causes of gastric ulcer by pyloric ligation are believed to be due to stress induced increase in gastric hydrochloric acid secretion and/or stasis of acid and the volume of secretion is also an important factor in the formation of ulcer due to exposure of the unprotected lumen of the stomach to the accumulating of acid.

The ligation of the pyloric end of the stomach causes accumulation of gastric acid in the stomach. This increase in the gastric acid secretion causes ulcers in the stomach. The lesions produced by this method are located in the lumen region of the stomach [23].

Ethanol induced gastric lesion formation may be due to stasis in gastric blood flow which contributes to the development of the haemorrhage and narcotic aspects of tissue injury [24]. Alcohol rapidly penetrates the gastric mucosa apparently causing cell and plasma membrane damage leading to increased intra cellular membrane permeability to sodium and water. The massive intracellular accumulation of calcium represents a major step in the pathogenesis of gastric mucosal injury. This leads to cell death and exfoliation in the surface epithelium [25].

In the present study, EECG showed protection against gastric lesions in the experimental rats, reduced gastric volume, free acidity, total acidity and ulcer index thus

showing the anti-secretory mechanism involved in the extracts for their anti-ulcerogenic activity. 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[26].

The protection of EECG against characteristic lesions may be due to both reductions in gastric acid secretion and gastric cycloprotein or enhancement of the mucosal barrier through the increase production of prostaglandin and this may be due to the presence of glycosides. Further studies are needed for their exact mechanism of action on gastric acid secretion and gastric cytoprotection.

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