



EVALUATION OF ANTI-ULCER ACTIVITY OF *OCHNA OBTUSATA* IN VARIOUS EXPERIMENTAL MODELS

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ABSTRACT

Ochna obtusata a member of the Ochnaceae family is used in folk medicine because of its treatment in ulcer, asthma and brinichitis. The purpose of the present study is to investigate the acute oral toxicity and anti-ulcer profile of the Ethanol Extract of *Ochna obtusata* (EOO) extract in albino rats. Study on acute toxicity of extract found to be safe at the doses 2000mg/kg body weight orally as per OECD guidelines No.423. EOO at the doses of 200 and 400 mg/kg body weight orally was administered to evaluate anti-ulcer activity by using Ethanol, indomethacin, pyloric ligation (PL), and cold-restraint stress induced gastric ulcer models in Albino rats. Ethanol Extract of *Ochna obtusata* dose dependent inhibition in ethanol induced gastric lesions, causing 69.49% protection at 400 mg/kg, and 54.68% protection at 200 mg/kg, EOO dose dependent inhibition in indomethacin induced gastric lesions, causing 59.64% protection at 400 mg/kg and 50.85% protection at 200 mg/kg, EOO dose dependent inhibition in pylorus ligation induced gastric ulcer (PL) causing 69.20% protection at 400 mg/kg and 48.04% protection at 200 mg/kg and it also dose dependent inhibition in Cold-restraint stress induced gastric lesions, causing 68.04% protection at 400 mg/kg, and 57.32% protection at 200 mg/kg. All the results are found to be statistically significant ($p \leq 0.05$). Hence we suggest that Ethanol Extract of the whole plant of *Ochna obtusata* possess anti-ulcer properties that may be due to cytoprotective mechanism. These results support the ethnomedical uses of the plant in the treatment of gastric ulcer.

Keywords:- *Ochna obtusata* , Anti-Ulcer activity.

INTRODUCTION

The exact pathogenesis of ulcer continues to elude scientists and medical researchers, but a common ground has been proposed. Ulcers are produced when any factor causes an imbalance between the protective factors (mucus and bicarbonate) and aggressive factors (acid and pepsin) in the stomach. Such factors could range from natural causes (gastric cancer), infections (*H. pylori*), lifestyle (drugsnon steriodal antiinflammatory agents, alcohol, stress and cigarette smoking). Current treatment of ulcers in developing countries has been largely suppression of pain, with little or no strategy aimed at a cure. Herbal medicine is fast emerging as an alternative treatment to available synthetic drugs for treatment of ulcer possibly due to lower costs, availability, fewer adverse effects and perceived effectiveness.

Development of tolerance and incidence of relapses and side effects on clinical evaluation make their efficacy arguable. This has been the basis for the development of new antiulcer drugs, which includes herbal drugs [1-4].

Ochna obtusata DC. (Family- Ochnaceae). Habit: Small trees up to 8 m tall. Trunk & Bark: Bark greyish, smooth; blaze pinkish. Branches and branchlets: Branchlets terete, lenticellate, glabrous. Leaves : Leaves simple, alternate, distichous; stipules caducous and leaving scar; petioles ca. 0.4 cm long, planoconvex, glabrous; lamina 16 x 5 cm, elliptic or elliptic-oblong to obovate, apex acute to rounded, base acute to rounded, margin serrate, shining above, chartaceous, glabrous beneath; midrib raised above; secondary_nerves

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ca. 12 pairs, ascending towards apex; tertiary_nerves slender, reticulo-percurrent. Inflorescence / Flower : Inflorescence axillary or lateral racemes; flowers yellow; pedicels up to 2.5 cm long. Fruit and Seed: Drupe, 3-5 distinct drupes seated on the enlarged disk; seeds 1 drupe. Distribution: South Asia; in the Western Ghats- South, Central and Maharashtra Sahyadris. The leaves and roots of *Ochna obtusata* is used for ulcer, asthma and bronchitis [5]. From the source of literature documentation and relevant traditional approaches on plant drugs, the present investigation was carried out to investigate the anti-ulcer profile of the Ethanol Extract of *Ochna obtusata* leaves (EOO) is being reported here.

MATERIALS AND METHODS

Plant material

The leaves of *Ochna obtusata* was collected from Tirumala hills, Tirupati, Andhra Pradesh. India. It was identified and authenticated by Prof. Madhava Chetty, K., Taxonomist, S.V. University, Tirupati, Andhra Pradesh, India. A voucher specimen has been kept in our laboratory for future reference.

Preparation of plant extract

The collected whole plant was dried at room temperature, pulverized by a mechanical grinder, sieved through 40 mesh. About 100g of powdered materials were extracted with Ethanol (90%) using soxhlet apparatus. The extraction was carried out until the extractive becomes colourless. The extracts is then concentrated and dried under reduced pressure. The solvent free semisolid mass thus obtained is dissolved in tween 80 and used for the experiment. The percentage yield of prepared extract was around 14.5% w/w.

Animals Used

Albino rats (180–200 g) of either sex were maintained in a 12 h light/dark cycle at a constant temperature 25 °C with free access to feed (Sai durga feeds and foods, Bangalore) and water. All animals were fasted prior to all assays and were allocated to different experimental groups each of 6 rats. Moreover the animals were kept in specially constructed cages to prevent coprophagia during the experiment. All experiments were carried out according to the guidelines for care and use of experimental animals and approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Ethical committee clearance was obtained from IAEC (Institutional Animal Ethics Committee) of CPCSEA.

Acute toxicity study

The acute toxicity of Ethanol extract of *Ochna obtusata* leaves was determined as per the OECD guideline no. 423 (Acute Toxic Class Method). It was observed that

the test extract was not lethal to the rats even at 2000mg/kg dose. Hence, 1/10th (200mg/kg) and 1/5th (400mg/kg) of this dose were selected for further study [6].

ANTI-ULCER ACTIVITY

Ethanol induced gastric ulcer

Animals were randomly divided into four groups each of 6 rats. Group I treated with 4% v/v aqueous tween 80 (10 ml/kg p.o), Group II & III treated with Ethanol extract of *Ochna obtusata* (200 and 400mg/kg p.o) respectively for 14 days and Group IV treated with Omeprazole (20 mg/kg p.o) were administered 30min prior to induction of gastric ulcer. On the 14th day, Gastric ulcers were induced with ethanol at a dose of 8ml/kg [7] administered to all groups by orally. The animals were anaesthetized 6 h with ether and stomachs were incised along the greater curvature and the ulcer index for each rat was taken as the mean ulcer score.

Indomethacin induced gastric ulcer

Animals were divided into four groups each of six rats. Group I treated with 4% v/v aqueous tween 80 (10 ml/kg p.o), Group II & III treated with Ethanol extract of *Ochna obtusata* (200 and 400mg/kg p.o) respectively for 14 days and Group IV treated with Omeprazole (20 mg/kg p.o) were administered 30min prior to induction of gastric ulcer. On the 14th day, Gastric ulcer were induced with indomethacin (40 mg/kg p.o) administered to all groups after fasting for 24 h. The animals were sacrificed 4 h after treatment with the ulcerogenic agent [8] to assess the antiulcer activity and ulcer index were examined on the dissected stomachs as described below.

Pyloric ligation induced gastric ulcer

Animals were divided into four groups each of six rats. Group I treated with 4% v/v aqueous tween 80 (10 ml/kg p.o), Group II & III treated with Ethanol extract of *Ochna obtusata* (200 and 400mg/kg p.o) respectively for 14 days and Group IV treated with Omeprazole (20 mg/kg p.o) were administered 30min prior to induction of gastric ulcer. On the 14th day, all groups rats were fasted 24 h prior to induction of gastric ulcer. Pyloric ligation was done by ligating the pyloric end of the stomach of rats 1 h after drug administration [9]. Animals were allowed to recover and stabilized in individual cage and were deprived of water during post-operative period. After 4 h of surgery, rats were sacrificed by cervical dislocation and ulcer index were examined on the dissected stomachs as described below.

Cold-restraint stress-induced ulcers

Animals were divided into four groups each of six rats. Group I treated with 4% v/v aqueous tween 80 (10 ml/kg p.o), Group II & III treated with Ethanol extract of *Ochna obtusata* (200 and 400mg/kg p.o) respectively for 14 days and Group IV treated with Omeprazole

(20 mg/kg p.o). On the 14th day, One hour after drug treatment, the experimental rats were immobilized by strapping the hind limbs on a wooden plank and kept for 1 h 30min, at temperature of 3–5 °C [10]. One hour later, the animals were sacrificed by cervical dislocation and ulcers were examined on the dissected stomachs as described below.

Measurement of ulcer index

The stomachs were excised and were examined for hemorrhagic lesions in glandular mucosa. Immediately after the animals were sacrificed, their stomachs were dissected out, cut along the greater curvature and the mucosa were rinsed with cold normal saline to remove blood contaminant, if any. The sum of the length (mm) of all lesions for each stomach was used as the ulcer index (UI), and the percentage of inhibition (%I) was calculated as described by Nguelefack et al. (2005) [11] using the following formula:

$$\%I = \frac{(USc - USt)}{USc} \times 100$$

Where USc = ulcer surface area in control and USt = ulcer surface area in treated animals.

Statistical analysis

The data were expressed as mean \pm standard error mean (S.E.M). The Significance of differences among the group was assessed using one way and multiple way analysis of variance (ANOVA). The test followed by Dunnett's test p values less than 0.05 were considered as significance.

RESULTS

Acute toxicity study

Acute toxicity study in which the animals treated with the Ethanol Extract of *Ochna obtusata* at a higher dose of 2000 mg/kg did not manifest any significant abnormal signs, behavioral changes, body weight changes, or macroscopic findings at any time of observation. There was no mortality in the above-mentioned dose at the end of the 14 days of observation.

Effect of Ethanol Extract of *Ochna obtusata* on gastric ulcer induced by Ethanol

The Ethanol Extract of *Ochna obtusata* showed

significant anti-ulcer effect against ulcers induced by Ethanol in a dose dependent manner. In ethanol induced ulcer model, Ethanol Extract of *Ochna obtusata* at a dose of 200 and 400 mg/kg body weight showed protective effect of 54.68 and 69.49 %, respectively, whereas Omeprazole showed protection index of 75.72% at a dose of 20 mg/kg body weight (Table -1).

Effect of Ethanol Extract of *Ochna obtusata* on gastric ulcer induced by Indomethacin

The Ethanol Extract of *Ochna obtusata* showed significant anti-ulcer effect against ulcers induced by Indomethacin in a dose dependent manner. In Indomethacin induced ulcer model, Ethanol Extract of *Ochna obtusata* at a dose of 200 and 400 mg/kg body weight showed protective effect of 50.85 and 59.64%, respectively, whereas Omeprazole showed protection index of 77.04% at a dose of 20 mg/kg body weight (Table -2).

Effect of Ethanol Extract of *Ochna obtusata* on gastric ulcer induced by pylorus ligation (PL)

The Ethanol Extract of *Ochna obtusata* showed significant anti-ulcer effect against ulcers induced by pylorus ligation in a dose dependent manner. In PL induced ulcer model, Ethanol Extract of *Ochna obtusata* at a dose of 200 and 400 mg/kg body weight showed protective effect of 48.04 and 69.20%, respectively, whereas Omeprazole showed protection index of 80.05% at a dose of 20 mg/kg body weight (Table -3).

Effect of Ethanol Extract of *Ochna obtusata* on gastric ulcer induced by Cold-restraint stress

The Ethanol Extract of *Ochna obtusata* showed significant anti-ulcer effect against ulcers induced by Cold-restraint stress in a dose dependent manner. In the gastric ulcer induced by Cold-restraint stress, Ethanol Extract of *Ochna obtusata* at a dose of 200 and 400 mg/kg body weight showed again significant activity. Ethanol Extract of *Ochna obtusata* at a dose 200 and 400 mg/kg body weight showed dose-dependent protective effect of 57.32 and 68.04% respectively, whereas Omeprazole showed protection effect of 74.84% at a dose of 20 mg/kg body weight, in both the above models. (Table-4)

Table 1. Effect of Ethanol Extract of *Ochna obtusata* (EOO) in ethanol (8 ml/kg) induced gastric ulcer in rats

Group	Design of Treatment	Ulcer Index	Percentage Inhibition (% I)
I	Control (4% v/v aqueous tween 80, 10 ml/kg b.w) p.o	20.39 \pm 1.24	---
II	EOO (200mg/kg b.w) p.o	9.24 \pm 0.14*	54.68
III	EOO (400mg/kg b.w) p.o	6.22 \pm 0.17**	69.49
IV	Omeprazole (20mg/kg b.w) p.o	4.95 \pm 0.19**	75.72

Data are represented as mean \pm S.E.M. Statistical analysis was done by one-way ANOVA followed by Dunnett's multiple comparison test. *P < 0.01 and **P < 0.001 as compared to control (n = 6 in each group). EOO = Ethanol Extract of *Ochna obtusata* b.w= Body weight.

Table 2. Effect of Ethanol Extract of *Ochna obtusata* (EOO) in indomethacin (40 mg/kg) induced gastric ulcer in rats

Group	Design of Treatment	Ulcer Index	Percentage Inhibition (% I)
I	Control (4% v/v aqueous tween 80, 10 ml/kg b.w) p.o	17.64 ± 0.49	---
II	EOO (200mg/kg b.w) p.o	8.67 ± 0.41*	50.85
III	EOO (400mg/kg b.w) p.o	7.12 ± 0.21**	59.64
IV	Omeprazole (20mg/kg b.w) p.o	4.05 ± 0.36**	77.04

Data are represented as mean ± S.E.M. Statistical analysis was done by one-way ANOVA followed by Dunnett's multiple comparison test. *P < 0.01 and **P < 0.001 as compared to control (n = 6 in each group). EOO = Ethanol Extract of *Ochna obtusata*

B.W=Body weight.

Table 3. Effect of Ethanol Extract of *Ochna obtusata* (EOO) in pylorus ligation Induced ulcer model.

Group	Design of Treatment	Ulcer Index	Percentage Inhibition (% I)
I	Control (4% v/v aqueous tween 80, 10 ml/kg b.w) p.o	20.36 + 0.32	-
II	EOO (200mg/kg b.w) p.o	10.58 + 1.22*	48.04
III	EOO (400mg/kg b.w) p.o	6.27 + 0.44**	69.20
IV	Omeprazole (20mg/kg b.w) p.o	4.06 + 0.22**	80.05

Data are represented as mean ± S.E.M. Statistical analysis was done by one-way ANOVA followed by Dunnett's multiple comparison test. *P < 0.01 and **P < 0.001 as compared to control (n = 6 in each group). EOO = Ethanol Extract of *Ochna obtusata*

B.W=Body weight.

Table 4. Effect of Ethanol Extract of *Ochna obtusata* (EOO) on Cold-restraint stress induced Gastric ulcer in Rats

Group	Design of Treatment	Ulcer Index	Percentage Inhibition (% I)
I	Control (4% v/v aqueous tween 80, 10 ml/kg b.w) p.o	14.27 + 0.14	-
II	EOO (200mg/kg b.w) p.o	6.09 + 0.12*	57.32
III	EOO (400mg/kg b.w) p.o	4.56 + 0.17**	68.04
IV	Omeprazole (20mg/kg b.w) p.o	3.59 + 0.47**	74.84

Data are represented as mean ± S.E.M. Statistical analysis was done by one-way ANOVA followed by Dunnett's multiple comparison test. *P < 0.01 and **P < 0.001 as compared to control (n = 6 in each group). EOO = Ethanol Extract of *Ochna obtusata*

B.W=Body weight.

DISCUSSION & CONCLUSION

The results of this study show that the Ethanol extracts from the leaves of *Ochna obtusata* exert protective effects against ethanol, indomethacin, pylorus ligation and cold restraint stress-induced gastric mucosal damage. The anti-ulcer effect of *Ochna obtusata* was tested against gastric lesions induced by ethanol, the experimental model related to lesion pathogenesis with production of reactive oxygen species. Reactive oxygen species are involved in the pathogenesis of ethanol-induced gastric mucosal injury in vivo [12]. *Ochna obtusata* prevented the mucosal lesions induced by ethanol. Results in the present study also indicate similar alterations in the anti-oxidant status after ethanol induced ulcers. The gastric mucosal protection against ethanol can be mediated through a number of mechanisms that include enhancement of the gastric mucosal defense through increase in mucus and / or

bicarbonate production, reducing the volume of gastric acid secretion or by simply neutralizing the gastric acidity [13].

EOO may either reduce the gastric acid secretion or enhance the barrier defence of the mucosal wall. EOO dose dependent inhibition in ethanol induced gastric lesions (Table -1). Histopathological studies suggest that the ethanol damage to the gastrointestinal mucosa starts with microvascular injury, namely disruption of the vascular endothelium resulting in increased vascular permeability, edema formation and epithelial lifting [14].

Their anti-ulcerogenic potency was tested against indomethacin-induced ulcer. Indomethacin is a cyclooxygenase inhibitor which suppresses gastroduodenal bicarbonate secretion, reduces endogenous prostaglandin

biosynthesis and disrupts the mucosal barrier as well as mucosal blood flow in animals [15]. It is also well known that prostaglandins synthesized in large quantities by the gastrointestinal mucosa can prevent experimentally induced ulcers by ulcerogens. Thus, when the ulcers lesions are induced by indomethacin, the cytoprotective effect of the anti-ulcer agent can be mediated through endogenous prostaglandins [16]. The results obtained show that the mean ulcer index was significantly reduced in the Ethanol extracts from the *whole plant* of *Ochna obtusata* treated groups, compared to their respective controls. *Ochna obtusata* extracts may be stimulate the secretion of prostaglandins or possess prostaglandins like-substances (Table -2).

In order to probe the effectiveness of *Ochna obtusata* extracts in preventing gastric ulcer and also assess their antisecretory activity, they were tested against pylorus ligation- and cool stress induced ulcer. Pylorus ligation- [17] and cold restrained stress- induced ulcers are results of auto digestion of the gastric mucosal barrier probably due to excess production and accumulation of HCl in the stomach. Gastric acid is an important factor for the genesis of ulceration in pylorus-ligated rats. The activation of the vagus-vagal reflux by stimulation of pressure receptors in the antral gastric mucosa in the hyper secretion model of pylorus ligation is believed to increase gastric acid secretion [18]. The current data clearly demonstrated that, EOO in a dose-dependent manner decreased hydrogenionic concentration suggesting that the pharmacological mechanism has a relationship to antisecretory activity (Table -3).

To further confirm its anti-ulcerogenic effect we have evaluated the efficacy of EOO against Cold-restraint stress -induced ulcer model. Gastric ulceration induced by

stress is probably mediated by the presence of acid, increase in gastric motility, [19] mast cell degranulation, decreased gastric mucosal blood flow [20], decreased prostaglandin synthesis [21] and augmented excretion of glycoproteins in the mucus. Moreover, stress-induced ulcer can be prevented partially or entirely by vagotomy; vagal over activity has been suggested to be the principal factor in stress-induced ulceration [22]. Any of these factors could play a role in genesis of stress-induced ulcers. Oral administration of the Ethanol extracts of *Ochna obtusata* showed dose dependent inhibition of gastric ulceration induced by Cold-restraint stress (Table -4).

The Ethanol extracts of *Ochna obtusata* at a dose of 400mg/kg showed similar activity to that of omeprazole (a proton pump inhibitor, which is used to heal stomach and duodenal ulcers). The gastro protective effect of omeprazole is mediated through block of acid secretion by inactivation of H⁺/K⁺-ATPase [23,24]. This study reveals that the aqueous and methanol extracts from the leaves of *Ochna obtusata* are potent inhibitors of gastric mucosal lesions caused by ethanol, indomethacin, pylorus ligation and cold-restraint stress in rats.

Further, our results fortify the ethano pharmacological importance of EOO as an anti-ulcer agent. Etiology of ulcers produced in different ulcer models is diverse. Since EOO has been found effective in various models depicting its anti-ulcerogenic activity. EOO and its active constituents may emerge as more effective therapeutic agent to counter gastric ulcer incidence. However more experimentation, detailed phytochemical and experimental analysis are required for a definitive conclusion.

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