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EVALUATION OF ANTISECRETORY ACTIVITY OF *IPOMOEA ERIOCARPA* IN ALBINO WISTAR RATS

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ABSTRACT

Ipomoea eriocarpa a member of the Convolvulaceae family is used in folk medicine because of its treatment in ulcer, fever and rheumatism. The purpose of the present study is to investigate the acute oral toxicity and anti-ulcer profile of the Petroleum Ether Extract of *Ipomoea eriocarpa* (PIE) 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. PIE 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. Petroleum Ether Extract of *Ipomoea eriocarpa* dose dependent inhibition in Ethanol, indomethacin, pyloric ligation (PL), and cold-restraint stress induced gastric lesions in rats. All the results are found to be statistically significant ($p \leq 0.05$). Hence we suggest that Petroleum Ether Extract of the whole plant of *Ipomoea eriocarpa*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: *Ipomoea eriocarpa*, Convolvulaceae, Ethanol, Indomethacin, Pyloric ligation (PL), and Cold-restraint stress.

INTRODUCTION

Peptic ulcer disease (encompassing gastric ulcer and duodenal ulcer) affect a large portion of the world population and are induced by several factors, including stress, smoking, nutritional deficiencies, and ingestion of non-steroidal anti-inflammatory drugs [1]. The patho physiology of these ulcers involves an imbalance between offensive (acid, pepsin, and *Helicobacter pylori*) and defensive factors (mucin, prostaglandin, bicarbonate, nitric oxide and growth factors). Today, there are two main approaches for treating peptic ulcer. The first deals with reducing the production of gastric acid and the second with re-enforcing gastric mucosal protection [2,3]. There has been a rapid progress in the understanding of the pathogenesis of peptic ulcer. Modern approach to this includes proton pump inhibitors, histamine receptor blockers, drugs affecting the mucosal barrier and prostaglandin analog [4]. 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.

Ipomoea eriocarpa R.Br. (Family: Convo lvulaceae) often called annual morningglories, are summer annual or perennial broadleaf plants. *Ipomoea eriocarpa* R.Br. are often cultivated as ornamentals, however, under favorable conditions they can become troublesome weeds. They are also a major agricultural weed problem in the San Joaquin Valley of California, where several species of *Ipomoea* are found. Control is critical from crop emergence to harvest. Destroy seedlings while they are small, because once they have twined up stems they are difficult to control without injuring the crop. Seeds remain viable in soil for long periods. Seeds of Ipomoea species contain many types of alkaloids, including some that are neurotoxins to humans and animals when consumed. Fortunately, there is typically

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not enough seed in contaminated grain to cause harm to livestock. Most seedlings emerge following irrigation, but they may also appear when surface soil is too dry to allow germination of other annuals. Cotyledons (seed leaves) are butterfly shaped and more deeply notched and much larger than those of field bindweed. First true leaves are heart shaped with deep lobes at the base. Mature plants have long stems that climb and twine. Leaves are large, heart shaped and/or three lobed, and are alternate to one another along the stem. Both leaf types can occur on the same plant. The funnel-shaped flower varies in color depending on the species, from violet or blue to pink and red. Fruit are pods that release seeds through slits. Seeds germinate down to a depth of 4 inches (10 cm) or more, much deeper than most annuals. The whole plant of *Ipomoea eriocarpa* is used for ulcer, fever and rheumatism [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 Petroleum Ether Extract of *Ipomoea eriocarpa* whole plant (PIE) is being reported here.

MATERIALS AND METHODS

Plant material

The whole plant of *Ipomoea eriocarpa* 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 40mesh. About 100g of powdered materials were extracted with petroleum ether (60°-80°C) 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 9.5%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 procedure was followed according to the OECD guidelines 423 (Acute toxic class method). The acute toxic class method is a step wise procedure with 3 animals of single sex per group. Depending on the mortality and or moribund status of the animals, on an average 2-4 steps may be necessary to allow judgment on the acute toxicity of the testing substance. According to this procedure minimum number of animals were to be used for acceptable data band scientific conclusion. The method uses defined doses (5, 50, 300, 2000 mg/kg body weight) and the results allow a substance to be ranked and classified according to the globally harmonized system (GHS) for the classification of chemical which causes acute toxicity.

Adult female wistar rats were used for this study. The starting dose of whole plant of *Ipomoea eriocarpa* extract was 2000 mg/kg body weight, as most of the crude extracts possess LD_{50} value more than 2000 mg/kg body weight. The dose was administered to overnight fasted rats and food was withheld for a further 3-4 hours after administration of the drug and observed for signs of toxicity.

Body weight of the rats before and after treatment were noted and any changes in skin, eye, and mucous membranes, salivation, nasal discharge, urination and behavioral (sedation, depression), neuromuscular (tremors, convulsions), cardiovascular, lethargy, sleep and coma were noted. The onset of toxicity was also noted. The animals were kept under observation for 14 days.

The acute toxicity of Petroleum Ether extract of *Ipomoea eriocarpa* whole plant 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, $1/10^{th}$ (200mg/kg) and $1/5^{th}$ (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 Pet Ether extract of *Ipomoea eriocarpa*(200and 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 14^{th} 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 Pet Ether extract of Ipomoea eriocarpa (200and 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 of 4% v/v aqueous tween 80 (10 ml/kg p.o), Group II & III treated with Pet Ether extract of Ipomoea eriocarpa (200and 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 Pet Ether extract of Ipomoea eriocarpa (200and 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 ± 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

The acute oral toxicity study was done according to OECD guidelines 423 (acute toxic class method). A single dose of 2000 mg/kg body weight/po of the PIE was administered to 6 female rats. Animals were observed for signs of toxicity for first 3 hours at 30 min time intervals. Thereafter animals were observed for 24 hours with continuous monitoring. The animals were observed for further 14 days period for all toxicity signs. There was no considerable change in body weight before and after treatment and no signs of toxicity were observed. LD50 cut off dose per kilogram body weight was categorized as X (unclassified). The results are shown in Table -1. Acute toxicity study in which the animals treated with the Petroleum Ether Extract of Ipomoea eriocarpa 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 Petroleum Ether Extract of Ipomoea eriocarpa on gastric ulcer induced by Ethanol

The Petroleum Ether Extract of *Ipomoea eriocarpa* showed significant anti-ulcer effect against ulcers induced by Ethanol in a dose dependent manner. In ethanol induced ulcer model, Petroleum Ether Extract of *Ipomoea eriocarpa* at a dose of 200 and 400 mg/kg body weight showed significant protective effect same like Omeprazole showed protection (Table -2).

Effect of Petroleum Ether Extract of Ipomoea eriocarpa on gastric ulcer induced by Indomethacin

The Petroleum Ether Extract of *Ipomoea eriocarpa* showed significant anti-ulcer effect against ulcers induced by *Indomethacin* in a dose dependent manner. In Indomethacin induced ulcer model, Petroleum Ether Extract of *Ipomoea eriocarpa* at a dose of 200 and 400 mg/kg body weight showed significant protective effect same like Omeprazole showed protection (Table -3).

Effect of Petroleum Ether Extract of Ipomoea eriocarpa on gastric ulcer induced by pylorus ligation (PL)

The Petroleum Ether Extract of *Ipomoea*

eriocarpa showed significant anti-ulcer effect against ulcers induced by pylorus ligation in a dose dependent manner. In PL induced ulcer model, Petroleum Ether Extract of *Ipomoea eriocarpa* at a dose of 200 and 400 mg/kg body weight showed significant protective effect same like Omeprazole showed protection (Table -4).

Effect of Petroleum Ether Extract of Ipomoea eriocarpa on gastric ulcer induced by Cold-restraint stress

The Petroleum Ether Extract of *Ipomoea eriocarpa* 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*, Petroleum Ether Extract of *Ipomoea eriocarpa* at a dose of 200 and 400 mg/kg body weight showed again significant activity. Petroleum Ether Extract of *Ipomoea eriocarpa* at a dose 200 and 400 mg/kg body weight showed dose-dependent significant protective effect same like Omeprazole showed protection effect (Table-5).

Table 1: Acute Oral Toxicity Study Petroleum Ether Extract of *Ipomoea eriocarpa*

S.No	Treatment	Dose	Weight of the animal in grams		Signs of toxicity	Onset of toxicity	Reversible or Irreversible	Duration
			Before test (1st day)	After test (14th day)				
1	PIE	2 g/kg	190	195	No signs of toxicity	Nil	Nil	14 days
2	PIE	2 g/kg	200	205	No signs of toxicity	Nil	Nil	14 days
3	PIE	2 g/kg	195	200	No signs of toxicity	Nil	Nil	14 days
4	PIE	2 g/kg	185	190	No signs of toxicity	Nil	Nil	14 days
5	PIE	2 g/kg	195	200	No signs of toxicity	Nil	Nil	14 days
6	PIE	2 g/kg	195	195	No signs of toxicity	Nil	Nil	14 days

Table 2: Effect of Petroleum Ether Extract of *Ipomoea eriocarpa (*PIE) *in* ethanol (8 ml/kg) induced gastric ulcer in rats

Group	Design of Treatment	Ulcer Index
I	Control (4% v/v aqueous tween 80, 10 ml/kg b.w) p.o	20.54 ± 1.46
II	PIE (200mg/kg b.w) p.o	11.33 ± 0.18*
III	PIE (400mg/kg b.w) p.o	7.95 ± 0.36**
IV	Omeprazole (20mg/kg b.w) p.o	6.65 ±0.19**

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). PIE = Petroleum Ether Extract of *Ipomoea eriocarpa*
b.w= Body weight.

Table-3: Effect of Petroleum Ether Extract of *Ipomoea eriocarpa (*PIE) *in* indomethacin (40 mg/kg) induced gastric ulcer in rats

Group	Design of Treatment	Ulcer Index
I	Control (4% v/v aqueous tween 80, 10 ml/kg b.w) p.o	17.08 ± 0.44
II	PIE (200mg/kg b.w) p.o	8.54 ± 0.27*
III	PIE (400mg/kg b.w) p.o	7.29 ± 0.21**
IV	Omeprazole (20mg/kg b.w) p.o	5.67 ± 0.33**

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). PIE = Petroleum Ether Extract of *Ipomoea eriocarpa*
B.W=Body weight.

Table-4: Effect of Petroleum Ether Extract of *Ipomoea eriocarpa (*PIE) in pylorus ligation Induced ulcer model.

Group	Design of Treatment	Ulcer Index
I	Control (4% v/v aqueous tween 80, 10 ml/kg b.w) p.o	21.44 + 0.32
II	PIE (200mg/kg b.w) p.o	12.52 + 1.22*
III	PIE (400mg/kg b.w) p.o	6.13 + 0.21**
IV	Omeprazole (20mg/kg b.w) p.o	4.57 + 0.12**

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). PIE = Petroleum Ether Extract of *Ipomoea eriocarpa*
B.W=Body weight.

Table -5: Effect of Petroleum Ether Extract of *Ipomoea eriocarpa (*PIE) on Cold-restraint stress induced Gastric ulcer in Rats.

Group	Design of Treatment	Ulcer Index
I	Control (4% v/v aqueous tween 80, 10 ml/kg b.w) p.o	12.47 + 0.67
II	PIE (200mg/kg b.w) p.o	5.67 + 0.16*
III	PIE (400mg/kg b.w) p.o	4.59 + 0.67**
IV	Omeprazole (20mg/kg b.w) p.o	3.50 + 0.17**

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). PIE = Petroleum Ether Extract of *Ipomoea eriocarpa*
B.W=Body weight.

DISCUSSION & CONCLUSION

The results of this study show that the Petroleum Ether extracts from the whole plant of Ipomoea eriocarpa exert protective effects against ethanol, indomethacin, pylorus ligation and cold restraint stress-induced gastric mucosal damage. The anti-ulcer effect of Ipomoea eriocarpa 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]. Ipomoea eriocarpa 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].

PIE may either reduce the gastric acid secretion or enhance the barrier defense of the mucosal wall. PIE dose dependent inhibition in ethanol induced gastric lesions (Table -2). 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 endogeneous prostaglandin biosynthesis and disrupts the mucosal barrier as well as mucosal blood flow in animals [15]. It is also well known that prostaglandins synthetized 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 endogeneous prostaglandins [16]. The results obtained show that the mean ulcer index was significantly reduced in the petroleum ether extracts from the whole plant of Ipomoea eriocarpa treated groups, compared to their respective controls. Ipomoea eriocarpa extracts may be stimulate the secretion of prostaglandins or possess prostaglandins like-substances (Table -3).

In order to probe the effectiveness of Ipomoea eriocarpa 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 ligature is believed to increase gastric acid secretion [18]. The current data clearly demonstrated that, PIE in a dose-dependent manner decreased hydrogenionic concentration suggesting that the pharmacological

mechanism has a relationship to antisecretory activity (Table -4).

To further confirm its anti-ulcerogenic effect we have evaluated the efficacy of PIE 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 petroleum ether extracts of Ipomoea eriocarpa showed dose dependent inhibition of gastric ulceration induced by Cold-restraint stress (Table -5).

The petroleum ether extracts of Ipomoea eriocarpa 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 whole plant of Ipomoea eriocarpa 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 PIE as an anti-ulcer agent. Etiology of ulcers produced in different ulcer models is diverse. Since PIE has been found effective in various models depicting its anti-ulcerogenic activity. PIE 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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