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Original Article

GASTROPROTECTIVE ROLE OF ETHANOLIC EXTRACT OF *POUZOLZIA WIGHTII BENN.* ON EXPERIMENTALLY INDUCED ULCER MODEL IN RATS

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ABSTRACT

The current study was carried out to examine the gastroprotective effects of ethanolic extract of *Pouzolzia wightii* against ethanol and pylorus ligation induced gastric mucosa injury in rats. Wistar albino rats were separated into 6 groups. Groups 1–2 were orally challenged with 2% tween 80; group 3 received 20 mg/kg omeprazole and 100 mg/kg cimetidine for ethanol and pylorus ligation model respectively, groups 4–6 received 100, 200 and 400 mg/kg of ethanolic seed extract, respectively. After 1 h, CMC or absolute ethanol was given orally to groups 2–6. After 5 h pretreatment pylorus ligation was induced to the rats of group 2-6. The injuries to the gastric mucosa were estimated through assessment of the gross appearance of ulcer areas, histology, enzymatic assays. Group 2 exhibited significant mucosal injuries, whereas reductions in mucosal injury were observed for groups 4–6. Groups 3–6 demonstrated reversal biochemical parameters like TBARS and GSH. Ethanolic extract of seeds of *Pouzolzia wightii* possess significant antiulcer activity through inhibition oxidative stress.

INTRODUCTION

Peptic ulcer disease (PUD) is a common problem of the gastro-intestinal tract with increasing incidence and prevalence attributed to various factors including the widespread use of non-steroidal anti-inflammatory drugs (NSAIDs). PUD is reported to affect about 14.5 million people worldwide with a mortality of about 4.08 million [1]. Although the etiology of gastric ulcers is still debated, it is accepted that gastric ulcers are triggered by an imbalance between factors that damage and those that protect the stomach. Mucosal damage, an initial step in gastric ulcer development, has been known to be due to hypersecretion of HCl through H^+, K^+-ATPase action [2], harbouring of *H. pylori* on the damaged mucin layer [3], the blockade of the cyclooxygenase enzyme system by NSAIDs [4], and oxidative stress by reactive oxygen species.

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Reactive oxygen species (ROS) and free radicals play an important role in the pathogenesis of several human diseases including GUD [5,6]. Various studies have also shown that the endogenous anti-oxidant defense enzymes play a principal role in eliminating ROS and free radicals generated from the action of factors that damage the stomach.

Pouzolzia wightii Benn is the shrub which is widely distributed in the Tirumala hills, Vijayanagaram, Mahaboobnagar district, etc... This is the dicot plant. This plant is monoecious; Stem type is erect or strong stems, tomentose. Leaf arrangement is opposite, alternate, leaf shape is lanceolate and ovate, Inflorescence is axillary, and flower type is sessile, male flowers are with calyx 1.5 mm across, perianth 4-lobed, free, inflexed, hairy at tip; bud truncate and female flowers are slightly shorter than male, perianth tubular.. Synonyms of the plant include karagada. Mainly leaves of this plant have uses like anti inflammatory, wound healing, ulcers, boils. It is not indigenous to this country but is rarely cultivated (7). The present study was designed to investigate antiulcer activity of *Pouzolzia wightii* in experimental animal model.

MATERIALS AND METHODS

Chemicals and drugs

Sodium lauryl sulfate, Potassium dihydrogen phosphate, sodium pyrophosphate, 5, 5 1 -dithio-bis-2-nitrobenzoic acid, trichloroacetic acid (TCA), thiobarbituric acid (TBA), sodium dihydrogen phosphate, Disodium hydrogen phosphate, were purchased from Sigma life sciences, Bangalore. All other chemicals used were of analytical grade with high purity.

Animals

Male Wistar albino rats (180 ± 20 g) were selected for the study. The animals were housed in clean polypropylene cages under hygienic and standard environmental conditions at 22°C ± 2°C, 12:12 h light: Dark cycle and 60 ± 5% relative humidity with free access to standard laboratory food and water ad libitum (SaiDurga Feeds and Foods, Bangalore). Mice were habituated to laboratory conditions for 1 week before the test. All the experiments were carried out during the light period (08:00-16:00) and conducted in accordance with the guidelines given by the committee for the purpose of control and supervision of experiments on animals (CPCSEA), New Delhi (India) and the Institutional Animal Ethics Committee approved the experimental protocol.

Plant material and preparation of extract

The leaves of *Pouzolzia wightii* was collected from the Tirumala forests, Tirupati, A.P, India in the month of July 2013 and was authenticated by Dr. K. Madhava Chetty, Professor and Head, Department of Botany, S. V. University, Tirupati. The leaves of *Pouzolzia wightii* was shade dried and coarsely powdered. The 500 g of the powdered plant material was defatted with petroleum ether (60-80°C) using a soxhlet extractor and then it is successively extracted with $CHCl_3$, Ethyl acetate and 70% ethanol each for 72 h and the extracts obtained from the solvents were filtered and concentrated using rota evaporator (Medika Instrument). The yield of the extracts was found to be 8.6%, 10.9% and 11.5%, respectively.

Preliminary phytochemical screening

All the extracts of *Pouzolzia wightii* were screened for the presence of carbohydrates, proteins, alkaloids, flavonoids, glycosides, triterpenoids, tannins and phenolic compounds, fats and fixed oils using the standard procedures [8].

Acute oral toxicity study

The acute oral toxicity study was performed according to the method described by Lorke. [9] Ethanolic extract when compared to other extracts up to a dose of 2000 mg/kg did not produce any signs of toxicity and mortality. Based on this the doses for ethanolic extract of *Pouzolzia wightii* (EEPW) for further experimental study were selected.

Antiulcer activity

Gastric Ulcer Induction by Ethanol

The animals were fasted for 24 h prior to the experiment [10]. Groups 1 and 2 received vehicle (2% tween 80) orally. Group 3 received an oral dose of 20 mg/kg omeprazole in 2% tween 80, and groups 4–6 received EEPW at doses of 100, 200 and 400 mg/kg BW p.o. as a pretreatment. At 1 h after pretreatment, the vehicle or absolute ethanol was orally administered to groups 2–7 [11]. One hour later, the rats were euthanized, and their stomachs were dissected.

Pylorus ligation (PL)-induced ulcers (12)

EEPW were administered for a period of 5 days by oral gavage and the rats were kept for 18 h fasting. Animals were anaesthetized using pentobarbitone (30 mg/kg, i.p.), the abdomen was opened and pylorus ligation was done without causing any damage to its blood supply. The animals were deprived of water during the post-operative period. After 6 h, stomachs were dissected out and cut open along the greater curvature and ulcers were scored in the glandular portion of the stomach. The volume of gastric juice (ml) and pH values were determined. The total acid secretion in the gastric juice supernatant was determined by titration to pH 7.0, using a 0.01 N NaOH solution, and phenolphthalein as indicator.

Macroscopic Gastric Lesion Evaluation

The rat stomachs were examined under a light microscope. The length and width (mm) of each individual hemorrhagic lesion was measured by a planimeter (10 × 10 mm^2= ulcer area) under a dissecting microscope (1.8×). The ulcer area (UA) was calculated using the sum of the areas of all lesions for each stomach [13]. The UA was calculated using the following formula:

Inhibition percentage (I%) was calculated as follows:

$$I\% = \left(\frac{UA_{control} - UA_{treated}}{UA_{control}} \right) \times 100$$

Antioxidant Activity

Preparation of homogenate

Gastric tissue homogenate 10% (w/v) was prepared in ice-cold 50 mM phosphate buffer (pH 7.4) containing a mammalian protease inhibitor cocktail and then centrifuged at 4,000 rpm for 10 minutes (4°C). The homogenates obtained were used for the estimation of Thiobarbituric acid reactive substance (TBARS), [14] reduced glutathione (GSH), [15].

Statistical analysis

Results were expressed as mean ± standard deviation and analyzed using Graph Pad Prism version 5.1 GraphPad Software, Inc using one-way analysis of variance followed by Dunnett's posttest. $P < 0.05$ was considered to be significant.

RESULTS

Preliminary phytochemical screening

The preliminary phytochemical analysis revealed the presence of alkaloids, carbohydrates, saponins, phenolic compounds, and flavonoids.

Table 1. Effect of EEPW on gastric content pH, gastric ulcer area and percentage inhibition of ethanol induced gastric mucosal lesions in rats

Treatment group	pH	Ulcer area	% Inhibition
Group 1	6.86±0.02	0	0
Group 2	2.89±0.06#	658±18.2#	0
Group 3	5.99±0.25**	164±20.8**	78
Group 4	5.62±0.09**	196±10.6**	63
Group 5	5.87±0.26**	98±11.2**	84
Group 6	6.02±0.74**	36±6.9**	92

Mean ± SEM. The data were analyzed by oneway ANOVA. # *P<0.01* vs Group 1; ***P<0.01* vs Group 2
Group 1: Rat received 2% tween 80 as vehicle control
Group 2: Rat received 2% tween 80 subjected to ethanol induced gastric lesion
Group 3: Rat received 20 mg/kg omeprazole subjected to ethanol induced gastric lesion
Group 4-6: Rat received EEPW (100, 200 & 400 mg/kg respectively) subjected to ethanol induced gastric lesion

Antiulcer activity of EEPW

The antiulcer activity of the EEPW in the ethanol-induced gastric lesion model is shown in Table 1. The results demonstrated that rats pretreated with omeprazole or EEPW prior to treatment with absolute ethanol (groups 3–6) exhibited significantly smaller areas of gastric ulceration than ulcer induced group 2. Absolute ethanol produced extensive and visible hemorrhagic lesions in the gastric mucosa. EEPW extract significantly inhibited the ulcer formation induced by absolute ethanol and obviously decreased the gastric mucosal damage in a dose-dependent manner, i.e., the EEPW significantly suppressed the formation of ulcers.

Table 2. Effect of EEPW on TBARS and GSH

Treatment group	TBARS µmol/g tissue	GSH nmol/mg tissue
Group 1	86.27±2.48	538±8.2
Group 2	215.63±0.76#	89±1.2#
Group 3	165.54±0.25**	394±2.8**
Group 4	115.22±0.19**	396±11.6**
Group 5	95.87±0.16**	498±14.2**
Group 6	86.02±0.24**	536±19.9**

Mean ± SEM. The data were analyzed by oneway ANOVA. # *P<0.01* vs Group 1; ***P<0.01* vs Group 2
Group 1: Rat received 2% tween 80 as vehicle control
Group 2: Rat received 2% tween 80 subjected to ethanol induced gastric lesion
Group 3: Rat received 20 mg/kg omeprazole subjected to ethanol induced gastric lesion
Group 4-6: Rat received EEPW (100, 200 & 400 mg/kg respectively) subjected to ethanol induced gastric lesion

The effect of EEPW on TBARS and GSH

The TBARS level was significantly higher in group 2 than in group 1, while groups 4–6 demonstrated significantly decreased TBARS level. In addition, the effect of the EEPW on the total GSH in gastric mucosal homogenates was assessed. Ethanol treatment caused a significant depletion of GSH in group 2 compared to group 1. In contrast, groups 4-6 exhibited significantly augmented GSH content (Table 2).

Histological Evaluation of Gastric Lesions

Histological observations of group 1 indicated that there was no disruption of the surface epithelium, while the histological examination showed extensive damage to the gastric mucosa in group 2, with necrotic lesions. Group 3-6 exhibited a mild disruption of the surface epithelium, with submucosal edema and leucocyte infiltration. These results demonstrated that the EEPW exerted cytoprotective effects (Figure 1).

Effect of EEPW on pylorus ligation induced gastric ulcers

The effect of five day treatment with EEPW and cimetidine on pylorus ligation induced gastric lesion in rats is shown in Table 3. Pretreatment with EEPW showed a dose-dependent antiulcer effect which was significant ($p < 0.05$) only at 400 mg/kg dose when compared with 2% tween 20 treated group. EEPW-treated group (100 and 200 mg/kg) demonstrated sig-

Table 3. Effect of EEPW on gastric pylorus ligation induced gastric ulcer in rats

Treatment group	pH	Gastric juice (ml)	Gastric acidity (mEq/l)
Group 1	5.26±0.62	0.34±0.24	6.32±1.02
Group 2	2.48±0.16#	0.98±0.42#	12.05±1.41#
Group 3	5.67±0.15**	0.65±0.70**	8.05±1.06**
Group 4	4. 52±0.19**	0.78±0.61**	10.02±0.98**
Group 5	5.27±0.26**	0.48±0.28**	7.06±0.89**
Group 6	5.92±0.84**	0.39±0.24**	6.88±1.12**

Mean ± SEM. The data were analyzed by oneway ANOVA. # *P<0.01* vs Group 1; ***P<0.01* vs Group 2
Group 1: Rat received 2% tween 80 as vehicle control
Group 2: Rat received 2% tween 80 subjected to pylorus ligation
Group 3: Rat received 100 mg/kg cimetidine subjected to pylorus ligation
Group 4-6: Rat received EEPW (100, 200 & 400 mg/kg respectively) subjected to pylorus ligation

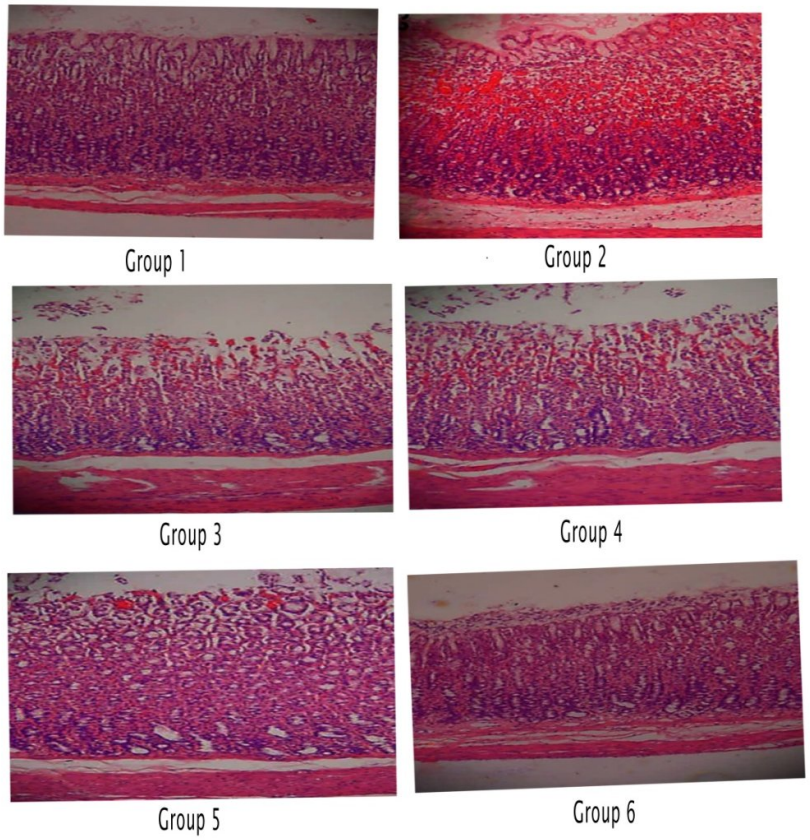

Figure 1. Effect of EEPW on gastric smooth muscle. Group 1: Rat received 2% tween 80 as vehicle control. Group 2: Rat received 2% tween 80 subjected to ethanol induced gastric lesion. Group 3: Rat received 20 mg/kg omeprazole subjected to ethanol induced gastric lesion. Group 4-6: Rat received EEPW (100, 200 & 400 mg/kg respectively) subjected to ethanol induced gastric lesion

nificant reduction ($p < 0.05$) in the volume of gastric acid secretion. Total acidity of gastric juice was also reduced, though not statistically significant. There was no significant alteration ($p > 0.05$) in pH of both the extract and cimetidine.

DISCUSSION

The current study investigated the gastroprotective activity of the ethanolic extract obtained from the *Pouzolzia wightii* seed in ethanol induced gastric lesions. EEPW presents a gastroprotective effect, reducing the lesions in the absolute ethanol induced gastric lesion model in a dose-dependent manner. In this model, the oxidative stress and the decrease in the GSH contribute to the gastric mucosa damages (16). It is well-established that the human consumption of ethanol depletes the NP-SH levels promoting a lipid peroxidation (17), and that the acute treatment with ethanol increases the oxidative stress.

Pylorus ligation is an important procedure that shows the possible changes of the parameters for gastric content e.g. volume of gastric juice, total acidity and pH [18]. Ulcers caused by pyloric ligation are due to increased accumulation of gastric acid and pepsin, leading to the autodigesion of gastric mucosa [19]. Inhibition of gastric acidity is one of the important protective factors, since overwhelming of the mucosal defense mechanisms by acid level leads to ulcer formation [20]. In this model, only 400 mg/kg of the extract exerted a significant reduction in ulcer index when compared with the control group. EEPW exerted a significant preventive antiulcer effect in the pylorus-ligated model as indicated by reduction in gastric volume at 200 and 400 mg/kg doses. These results suggest that the extract interfered with digestive effect of accumulated gastric juice. Gastric acid secretion is stimulated by histamine release from enterochromaffin-like cells in the oxyntic glands; gastrin, released from G cells in the pyloric gastric glands and by acetylcholine, released from postganglionic enteric neurons [21]. The reduction of gastric acidity and gastric secretory volume could be attributed to antihistamine effect, since antihistamine drugs like cimetidine blocks H_2receptors in the stomach thereby reducing the acidity of the gastric juice [20].

CONCLUSION

From these findings, it has been concluded that ethanolic extract of seeds of *Pouzolzia wightii* possess significant antiulcer activity through inhibition oxidative stress.

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