

ANTIULCER ACTIVITY OF *Pterospermum acerifolium*(L.) WILLD. STERCULIACEAERASIKA D. BHALKE^{1*}, SEEMA A. GOSAVI¹, SARITA S. PAWAR¹, RAOSAHEB S. SHENDGE¹, AND SUBODH C. PAL²¹Department of Pharmacognosy, Sanjivani College of Pharmaceutical Education and Research, Kopargaon, Maharashtra, ²N. D. M. V. P. S's College of Pharmacy, Nashik, (MS), India. Email: rasikabhalke@yahoo.co.in

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ABSTRACT

Objective: *Pterospermum acerifolium* has long been used in folk medicine in treatment ulcer. Therefore, Present study was designed to investigate the antiulcer effect of total methanolic extract of leaves, bark and wood of *Pterospermum acerifolium* (TMPAL, TMPAB and TMPAW).

Methods: antiulcer activity was assessed by using different models of gastric ulceration in rats. Acute gastric ulceration in rats was produced by oral administration of various noxious chemicals including aspirin or ethanol. Anti-secretory studies were undertaken using pylorus-ligated technique. Gastric total acid output was estimated in the pylorus ligated rats. TMPAW, TMPAB and TMPAL were administered in the dose of 200 mg/kg orally in all experiments. Omeprazole, ranitidine were used as a reference drug.

Results: In aspirin induced model TMPAW, TMPAB and TMPAL at the dose of 200 mg/kg and omeprazole at 20mg/kg produced a significant ($p < 0.001$) reduction in the ulcer index and has protection index of 65.94 %, 73.18, 51.78 and 86.54 % respectively. TMPAB at dose of 200 mg/kg and ranitidine at 20mg/kg has shown significant ($p < 0.001$) reduction in ulcer index upto 52.67 and 36.66 with protection index of 60.55% and 72.53% respectively in ethanol induced ulcer model. In pylorus-ligated rats, TMPAB produced a significant ($p < 0.001$) decrease of gastric juice volume; and significantly inhibited gastric acid. This inhibition was less than that of ranitidine (20 mg/kg).

Conclusion: Overall, TMPAB has shown a substantial and significant protection against gastric ulcers in all the models. This protective effect might have been mediated by both anti-secretory and cytoprotective mechanisms.

Keywords: Antiulcer activity, *Pterospermum acerifolium*, Total acid output, Aspirin induced ulcer, Ethanol induced ulcer and Pylorus ligation.

INTRODUCTION

Pterospermum acerifolium (L) Willd (Family: Sterculiaceae) commonly known as 'Dinner plate tree' is a large deciduous tree widely distributed in North Canada and in many parts India [1, 2]. In traditional system of medicine, the flowers are used as a general tonic, anti-tumor agent, and analgesic and for the treatment of diabetes, gastrointestinal disorders, leprosy, blood troubles, bronchitis, cough, cephalic pain, migraine and inflammation. Manna et al., have reported antiulcer and wound healing activity of bark in experimental animals [3, 4]. Muhit et al., have reported antioxidant activity of the bark [5].

The bark contains Kaempferol, kaempferol-3-O-galactoside, luteolin-7-O-glucoside, luteolin-7-O-glucuronide, kaempferide-7-O-beta-D-glucopyranoside, D-galactouronic acid, D-galactose and L-rhamnose. Flowers contain 24-beta ethylcholest-5-in-3-beta-O-alpha-cellobioside, 3, 7-dimethyl-7-methyl:Spentacosanolide, n-hexacosan-1, 26-diol-dilignoserate, β -amyrine, β -sitosterol and a mixture of acids and saturated hydrocarbons. The seeds contain palmitic, stearic, arachidic, behenic, myristic, lignoceric, oleic, linoleic, linolenic acids. Trunk bark and seeds gave the amino acids tyrosin, cystine, glycine, alanine and leucine [6-8].

Though various parts of *P. acerifolium* have been extensively used in the folklore medicine for treatment ulcer, there is no scientific evidence available for such activities. Hence, it is considered worthwhile to study the antiulcer property of TMPAW, TMPAB and TMPAL. In the present study, we report the antiulcer activity of TMPAW, TMPAB and TMPAL using aspirin induced ulcer, ethanol induced ulcer and pylorus ligated method.

MATERIALS AND METHODS

Animal used

Albino Wistar rats of either sex weighing between 150-250g were used. Animals were housed under standard conditions of temperature ($24 \pm 2^\circ\text{C}$) and relative humidity (30-70%) with a 12:12 light: dark cycle. The animals were given standard diet and water *ad libitum*. All procedures involving animals were carried out under the institute ethics committee approval.

Antiulcer activity

Aspirin induced ulcer

Animals were divided in groups of six animals each. Group I served as negative control received distilled water, Group II served as positive controls and received omeprazole at the dose of 20 mg/kg, and animals of group III, IV and V received TMPAW, TMPAB and TMPAL at the dose of 200 mg/kg, orally daily, respectively, for five days for ulcer protective studies. Aspirin in dose of 20 mg/kg was administration to the animals on the day of the experiment and ulcers were scored after 4 h. The animals were sacrificed and the stomach was then excised and cut along the greater curvature, washed carefully with 5.0 ml of 0.9 % NaCl and ulcers were scored by a person unaware of the experimental protocol in the glandular portion of the stomach. Ulcer index was then calculated by adding the total number of ulcers per stomach and the total severity of ulcers per stomach [10-12].

A score for the ulcer was made as: 0.5-Hemorrhage, 1-Streaks, 2-Spot ulcer, 3-Sever ulcer or Sever steaks, 4-Erosions, 5-Perforation.

Mean ulcer score for each animal was expressed as ulcer index. The percentage of

Ulcer protection was determined as follows:

$$\% \text{ Protective} = \frac{\text{Control mean ulcer index} - \text{test mean ulcer index}}{\text{Control mean ulcer index}} \times 100$$

Ethanol induced ulcer

The gastric ulcers were induced in rats of either sex weighing between 130-150 g by administrating absolute ethanol (8 ml/kg). They were kept in specially constructed cages to prevent coprophagia during and after the experiment. The rats were divided into groups each containing six animals and fasted for 24 h and allowed free access to water. The first group received control vehicle only and the second group received standard ranitidine in the dose of 20 mg/kg, group III, IV and V received TMPAW, TMPAB and TMPAL at the dose of 200 mg/kg, orally daily respectively, for five days for ulcer protective studies. On the sixth day of experiment the drugs were administered orally 30 min prior to the oral administration of absolute ethanol. The animals were anaesthetized

6 h latter with ether and stomach was incised along the greater curvature and ulceration was scored. The number of ulcers and the length of each ulcer were measured. A score for the ulcer was made as mentioned above [13-16].

Pyloric ligation method

In this method albino rats were fasted in individual cages for 24 h. Group I served as negative control received distilled water, Group II served as positive controls received ranitidine (20 µg/kg, p.o), and animals of group III, IV and V received TMPAW, TMPAB and TMPAL at the dose of 200 mg/kg, orally daily respectively, 1 h before pylorus ligation. Under light ether anesthesia, the abdomen was opened and the pylorus was ligated. The abdomen was then sutured. At the end of 4 h after ligation, the animals were sacrificed with excess of anesthetic ether, and the stomach was dissected out. Gastric juice was collected and its volume was measured. The glandular portion was then exposed and examined for ulceration [16]. Ulcer index was determined.

Estimation of total acid output

Total acid output of the gastric juice was estimated by titration of 0.1 ml of gastric juice with 0.01 N sodium hydroxide using phenolphthalein as indicator. Total acid output was expressed as mEq/L per 100 gm of body weight [16].

Statistical analysis

Mean values \pm S. E. M. were calculated for each parameter. For the determination of significant intergroup differences, each parameter was analyzed separately and one-way analysis of variance (ANOVA) was carried out. $p < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Ulcer index parameter was used for the evaluation of anti-ulcer activity since ulcer formation is directly related to factors such as reduction in gastric volume, decrease in free and total acidity. TMPAW, TMPAB and TMPAL at the dose of 200 mg/kg have decreased the intensity of gastric mucosal damage induced by ulcerogenic agents.

TMPAW, TMPAB and TMPAL at the dose of 200 mg/kg and omeprazole at 20mg/kg produced a significant ($p < 0.001$) reduction in the ulcer index (fig. 1) and has protection index of 65.94 %, 73.18, 51.78 and 86.54 % respectively as shown in table 1. Aspirin has been reported to produce ulcers by both local and systemic effects [17]. Aspirin causes direct irritant effect and mucosal damage by interfering with prostaglandin synthesis, increasing acid secretion by increasing the H^+ ion transport/back diffusion of H^+ ions, resulting overproduction of leukotrienes and other products of 5-lipoxygenase pathway. It decreases mucin, surface active phospholipids bicarbonate secretion, mucosal proliferation and also produces damage by formation of free radicals [18]. The possible protective effect of TMPAW, TMPAB and TMPAL against aspirin-induced gastric lesions could be due to prevention of direct irritation and due to its 5-lipoxygenase inhibitory effect.

TMPAW, TMPAB and TMPAL showed the ability to reduce significantly ($p < 0.001$) the severity of ulceration of stomach induced by absolute ethanol. Among all the extracts TMPAB at dose of 200 mg/kg and ranitidine at 20mg/kg has shown significant ($p < 0.001$) reduction in ulcer index upto 52.67 and 36.66 with protection index of 60.55% and

72.53% respectively as shown in table 1 and fig. 2. The results of histopathological investigation revealed that the pretreatment with TMPAB and ranitidine absolutely prevented the ethanol-induced congestion, hemorrhage, edema, necrosis, inflammatory and erosions and ulceration in the gastric mucosa of rats. The stomach appearance was normal. The incidence of ethanol-induced ulcers predominant in the glandular part of stomach was reported to stimulate the formation of leukotriene C4 (LTC4), mast cell secretory products [19], and reactive oxygen species resulting in the damage of rat gastric mucosa [20]. In ethanol model, ulcers are caused due to perturbations of superficial epithelial cells, notably the mucosal mast cells leading to the release of the vasoactive mediators including histamine, thus causing damage to gastric mucosa [21]. Mucosal blood flow has been attributed to be an important factor in the damage caused by alcohol and is modulated by prostaglandin [22]. Ethanol causes direct chemical damage, independent of acid secretion, to the surface epithelium as well to the microvascular apparatus, leading to increased vascular permeability and decrease in mucosal blood flow which is followed by hypoxia and hemorrhagic ulcer. Ethanol also lowers cellular glutathione level which by decreasing prostaglandin biosynthesis affects the natural gastroprotection. Ethanol also causes gastric damage by increasing the formation of leukotrienes and by generating ROS. Ethanol also induces gastric epithelial cell apoptosis triggered by the enhancement of mucosal TNF- α . It appears that TMPAB blocks ethanol induced gastric damage by modulating these phenomena. The results are presented in table 1.

Gastric secretion was evaluated as gastric juice volume and total acidity for 4 h after pylorus ligation. In pylorus-ligated rats, TMPAB produced a significant ($p < 0.001$) decrease of gastric juice volume; and significantly inhibited gastric acid. This inhibition was less than that of ranitidine (20 mg/kg) as shown in table 2. The reduction in total acidity, measured after pylorus ligation, suggest that the protective mechanism of the extract on gastric mucosa might involve an inhibition of gastric secretion. Pylorus ligation of rats for 6h resulted in accumulation of gastric secretory volume, and increase in titrable acidity and ulceration (Table 2). TMPAB has also showed significant effectiveness ($P < 0.05$) in pylorus ligation induced gastric ulcer model fig.3. It shows protection index of 71.31% at the dose of 200 mg/kg whereas standard drug ranitidine at 20mg/kg has shown 73.25 % protection. Total acidity of TMPAB treated group was found to be 27 mEq/L, standard ranitidine treated group 22 mEq/L which is less than that of negative control group which showed 56 mEq/L total acidity. Pylorus ligation induced gastric ulcers occur because of an increase in acid-pepsin accumulation due to pyloric obstruction and subsequent mucosal digestion and breakdown of the gastric mucosal barrier. A copious amount of mucus is secreted during superficial damage and provides favorable microenvironment in repair. Hence estimation of acid secretion, pepsin secretion and mucus secretion is a valuable part of the study to clarify the mechanism of action of the drug under trial [23-26].

Overall, TMPAB has shown a substantial and significant protection against gastric ulcers in all the models. This protective effect might have been mediated by both anti-secretory and cytoprotective mechanisms. Moreover, further insight into the precise mechanism of action is essential to exploit the complete potency of TMPAB and increase its usage in contemporary medicine.

Table 1: Anti-ulcer activity of TMPAW, TMPAB and TMPAL on different ulcer-induced models

Treatment	Aspirin induced ulcer		Ethanol induced ulcer		Pylorus ligated ulcer	
	Ulcer index	% protection	ulcer index	% protection	Ulcer index	% protection
Vehicle	144.83 \pm 4.045	--	133.50 \pm 7.143	--	129.00 \pm 3.222	--
TMPAW	49.33 \pm 3.018***	65.94	95.16 \pm 2.81***	28.71	95.00 \pm 3.141***	26.35
TMPAB	38.83 \pm 2.774***	73.18	52.667 \pm 3.997***	60.55	37.00 \pm 1.528***	71.31
TMPAL	69.83 \pm 3.301***	51.78	118.83 \pm 4.246	10.99	83.167 \pm 2.315***	35.53
Ranitidine 20mg/kg	§	§	36.667 \pm 1.498***	72.53	34.5 \pm 2.487***	73.25
Omeprazole 20mg/kg	19.5 \pm 1.118***	86.54	§	§	§	§

Values are mean \pm S.E.M. ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ compared to control group. One way ANOVA followed by Dunette's test.

Table 2: Effect of TMPAW, TMPAB and TMPAL on Gastric volume and total acidity of pylorus ligation induced-ulcer

Group	No. of Animals used	Gastric Juice Volume (ml) mean ± S.E.M.	Total Acidity mEq (H ⁺)/L
Control	6	3.9±0.019	56
Ranitidine 20mg/kg	6	2.01±0.0413*	22
TMPAW 200 mg/kg	6	3.2±0.13*	42
TMPAB	6	2.9±0.19*	27
TMPAL	6	3.7±0.38	37

Values are mean ± S.E.M. *p<0.05 significant as compared to control group.

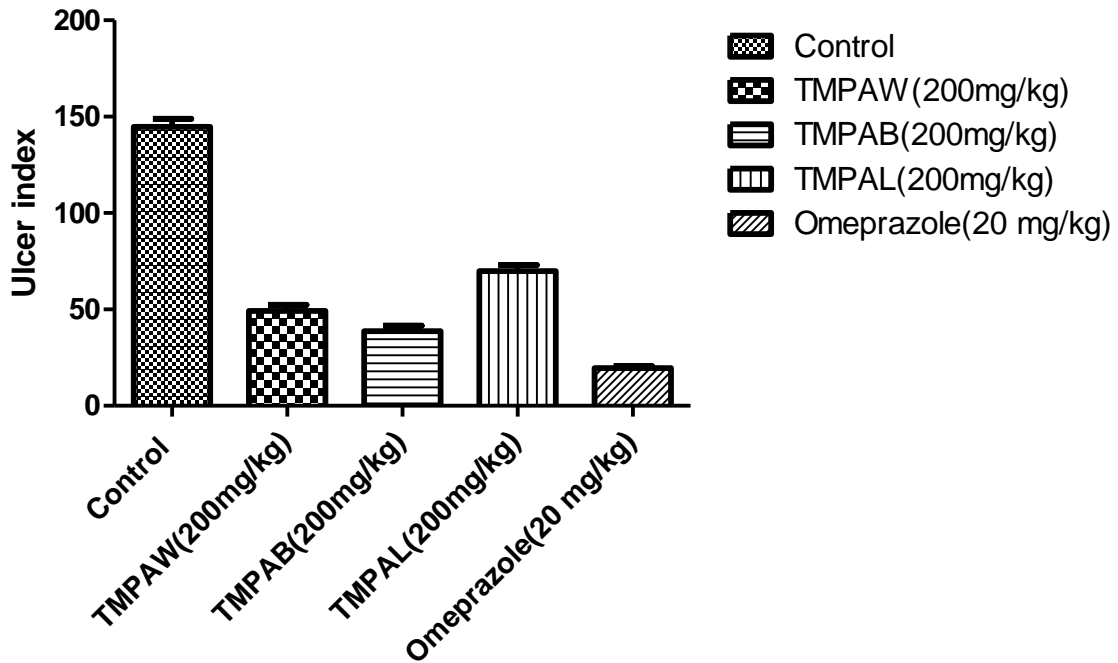


Fig.1: Effect of various extracts of *Pterospermum acerifolium* on aspirin-induced gastric ulcer. Data are expressed as means + SEM (n=6).

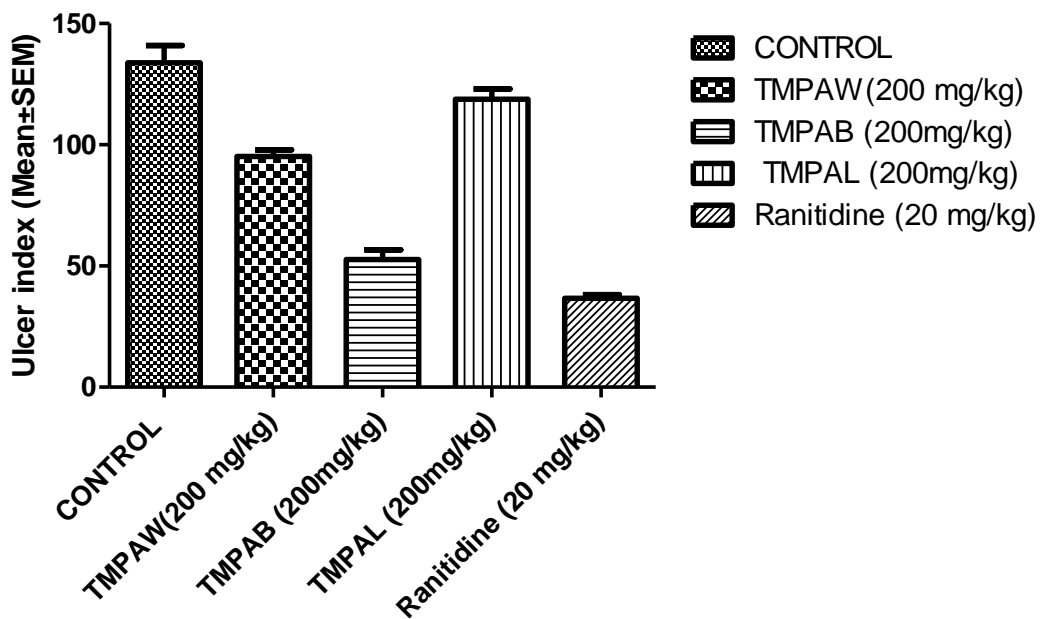


Fig. 2: Effect of various extracts of *Pterospermum acerifolium* on ethanol induced gastric ulcer. Data are expressed as means + SEM (n=6).

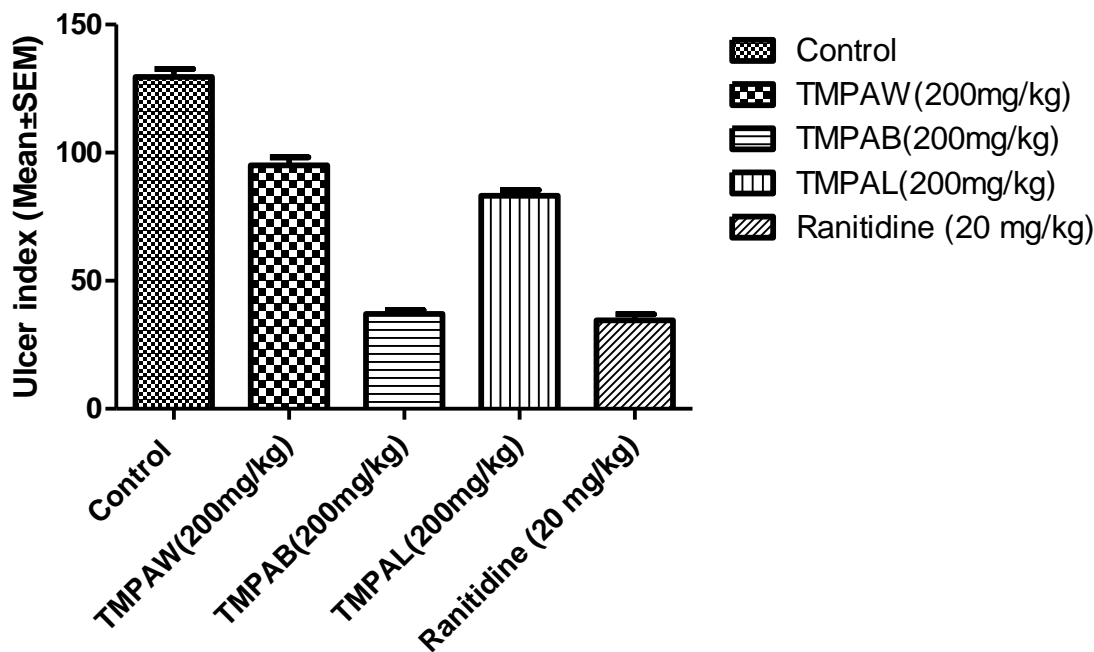


Fig. 3: Effect of various extracts of *Pterospermum acerifolium* on pylorus ligated method induced gastric ulcer. Data are expressed as means + SEM (n=6).

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