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Anti Ulcer activity of *Pterospermum acerifolium* (L) Willd leaves and its combined effect with H_2 Blocker and Proton Pump Inhibitor

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Abstract

The present study was performed to evaluate the anti-ulcerogenic activity of methanolic extract of *Pterospermum acerifolium* leaves by pyloric ligation described by shay *et al* in rats. Effect of different doses of methyl extract of *Pterospermum acerifolium* leaves, famotidine and omeprazole on volume of gastric secreation, pH, free acid, total acid and ulcer index were observed. In addition, the effect of low dose of methanolic extract of *Pterospermum acerifolium* leaves in combination with low dose of famotidine and omeprazole on the above parameters were studied. Methanolic extract of *Pterospermum acerifolium* (200 and 400mg/kg), famotidine (4mg/kg) and omeprazole (4mg/kg) produced significant antiulcer effect. Low doses of famotidine, omeprazole and Methanolic extract of *Pterospermum acerifolium* did not alter above parameter significantly. Combined administration of low dose of famotidine, omeprazole with Methanolic extract of *Pterospermum acerifolium* shows significant antiulcer effect where the reduction of gastric secreation, acidity and ulcer index with simultaneous increase in gastric pH.

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Methanolic extract of Pterospermum acerifolium leaves, famotidine and omeprazole, gastric ligation.

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INTRODUCTION

Peptic ulcer is a common disorder of the gastrointestinal system and the pathogenesis of peptic ulcer disease is multifactorial, including

Helicobacter pylori, gastric acid, pepsin, gastroduodenal motility, smoking, use of nicotine, and complex interaction between so-called aggressive and protective factors [1]. Mast cells are initiators and regulators of inflammation. After mast cell degranulation, histamine causes the secretion of gastric acid by triggering H_2-receptors, marked infiltration of inflammatory cells into the gastric mucosa, and expression of cytokines by triggering the H_1-receptors. Consequently, mast cells are considered as important effectors cells in the pathogenesis of gastritis, especially in *H pylori*-associated peptic ulcer [2]. Histamine H_2-receptor antagonists that possess a potent antisecretory activity can greatly enhances the healing rate of peptic ulcers. However, after H_2-receptor antagonist therapy peptic ulcer recurs rapidly and frequently. The possible reasons why the recurrence rate is high after H_2- antagonist therapy are acid rebound after the cessation of treatment, deficiency in gastric defensive factors such as gastric prostaglandin levels, and low maturity of regenerated mucosa.

The plant *Pterospermum acerifolium* (Common name Muchkunda) is used in traditional medicines for its haemostatic and wound healing properties distributed in tropical Asia. Initial pharmacological screening also shows the presence of anti-inflammatory, analgesic, antioxidant, antiulcer, wound healing and antipyretic properties [3-8]. Therefore, the present study was undertaken for the first time to investigate combined activity of methanol extract of *Pterospermum acerifolium* (MEPA) with famotidine and omeprazole in gastric ulceration by gastric ligation method in rats.

MATERIAL AND METHODS

Plant Collection

The leaves of *Pterospermum acerifolium* (L) willd were collected from Asansol, West Bengal, India in September 2010. A herbarium sheet was prepared and it was identified and authenticated by the Botanical survey of India, Howrah, and West Bengal, India. The leaves were dried in shade to avoid the deterioration of phytoconstituents and made into a coarse powder by using a grinder. Herbarium sheet was submitted to Shibpur Botanical Garden, Botanical survey of India,

Howrah, West Bengal, India, under specimen number CNH/I-I/ (289)/2008/Tech.II/331 for authentication. The specimen has been identified and authenticated as *Pterospermum acerifolium* (L) willd. (Figure1)

Fig.1. Picture of *Pterospermum acerifolium* (L) willd leaves.

Preparation of extract

The air dried crushed leaves (1000g) were soaked for 12 hr in Methanol (3L) at room temperature. The residue was extracted with hot Methanol under reflux 3 times (each 1500 ml) after vacuum filtration. All solvent was evaporated under vacuum and extract was then lyophilized, to yield approximately 12% w/w/) of the residue, which was stored at 20°C until use. The concentrate was suspended in 5% w/v Tween 80 and given at dose 1ml/100gm body weight.

Drugs used

Fresh solutions of famotidine (Lupin, Bhopal) and omeprazole (Cipla, Sikim) were prepared daily in DMSO.

Treatment of animals

Healthy male and female rats (Wistar albino) of 4-8 weeks old were selected after physical and behavioural veterinary examination from Institutional Animal House of Gupta College of Technological Sciences. The weight range was fall within ± 20% of the mean body for each sex at the time of initiation of treatment. All experiments involving animals complies with the ethical standards of animal handling and approved by Institutional Animal ethics committee (955/A/06/CPCSEA). All the selected animals were kept under acclimatization on the same day. The animals were acclimatized for minimum 5 days before initiation of dosing. The rats were housed in standard polypropylene cages with stainless steel top grill ingroup of 6 rats per cage. Clean autoclaved paddy husk was used as bedding. The paddy husk was changed at least thrice in a week. The animals were kept in a clean environment with 12-hour light and 12-hour dark cycles. The air was conditioned at 22±3oC and the relative humidity was maintained between 30-70% with 100% exhaust. Standard rat pellet feed was provided *ad libitum* throughout the study, except over night fasting prior to blood collection and was offered the feed immediately after completion of blood collection of all the animals. Drinking water was provided *ad libitum* in polypropylene bottles with a stainless steel sipper tube throughout study period.

Gastric Ligation method

Rats were divided into various groups (n=6 in each group) *viz.,* Control, dimethylsulphoxide (DMSO) (vehicle) treated, famotidine treated (1 and 4 mg/ kg), omeprazole treated (1 and 4 mg/kg), MEPA (200 and 400 mg/kg) and finally a combination treatment of MEPA (200 mg/kg) with famotidine (1 mg/kg) and MEPA (200 mg/kg) with omeprazole (1mg/kg). All drugs were administered intraperitoneally (i.p.) 1 h prior to pyloric ligation (PL), except MEPA which was given 2 h prior to PL orally and two groups treated with DMSO - one receiving DMSO 4 h prior to PL and the other 1 h prior to PL. Control group received normal saline. The volume of all the above injections varied between 0.2 to 0.4 ml.

Pyloric ligation was performed as described by Shay *et al.*[9] Rats were fasted for 36 h prior to the surgical procedure and kept in raised mesh-bottomed cages to avoid coprophagy. Under urethane anesthesia (80 mg/100 g, i.p.) the abdomen was opened by a small midline incision below the xiphoid process. The pyloric portion of the stomach was identified, slightly lifted out and ligated, avoiding traction to the pylorus or damage to the blood supply. The stomach was then replaced carefully and the abdominal wall closed by interrupted sutures. Animals were deprived of both food and water during the postoperative period and were sacrificed at the end of 19- 20 h after the operation. The stomach was dissected out as a whole by passing a ligature at the esophageal end.

The stomach was separated from the surrounding tissues and organs and thus brought out as a whole along with its contents. The contents were subjected to centrifugation (3000 rpm for 10 min) and then analyzed for volume, pH, and free and total acidity.
The pH was estimated using Indikrom pH strips (Glaxo India Limited, India) with pH ranges of 2.0-4.5 and 5.0-8.5 with a difference in range of 0.5. Free acidity and total acidity were estimated by titrating 1 ml of the centrifuged sample with 0.01 N NaOH, using Topfer's indicator and phenolpthalein indicator, respectively. Acidity was expressed in clinical units, i.e the amount of 0.01 N NaOH base required to titrate 100 ml of gastric secretion. For estimation of ulcer index, the stomach was cut open along the greater curvature and the inner surface was examined for ulceration with the help of a simple dissecting microscope. Usually, circular lesions were observed but, sometimes, linear lesions were also

seen. The ulcer index was calculated by using the formula.[10]

$$\text{Ulcer index} = \frac{10}{X} \quad \text{where, } X = \frac{\text{Total mucosal area}}{\text{Total ulcerated area}}$$

Histological evaluation

Specimens of the gastric walls from each rat were fixed in 10% buffered formalin and processed in a paraffin tissue processing machine. Sections of the stomach were made at a thickness of 5 µ and stained with hematoxylin and eosin for histological evaluation.

Statistical analysis

Results are expressed as the mean value ± standard error of mean (S.E.M.). Within group comparisons were performed by the analysis of variance using ANOVA test. Significant difference between control and experimental groups was assessed by student's ttest. A probability level of less than 5 %($P < 0.05$) was considered significant.

RESULTS

Effect of DMSO (vehicle)

The DMSO administered prior (4 h or 1 h) to pylorus ligation did not produce any significant change in the volume of gastric secretion, pH, free acid, total acid and ulcer index as compared to the control values.

Effect of famotidine

Famotidine in a dose of 1 mg/kg did not produce any significant change in any of the parameters studied. [Table - 1] However 4 mg/kg famotidine produced significant decrease in the volume of gastric secretion, free acid, total acid and ulcer index along with significant (P <0.01) increase in the pH as compared to the control.

Effect of Omeprazole

Omeprazole in a dose of 1 mg/kg produced a significant (P <0.05) decrease in the volume of gastric secretion as compared to the control value without significantly modifying the pH, free acid, total acid and ulcer index values. [Table - 1] Whereas, omeprazole in doses of 4 mg/kg produced significant (P <0.01) dose-dependent increase in pH value and significant dose-dependent decrease in other parameters, as compared to the control values. [Table - 1]

Effects of MEPA

MEPA in a dose of 200 mg/kg did not modify any of the parameters significantly. However, in doses of 400 mg/kg, it produced a dose-dependent decrease in the volume of gastric secretion, free acid, total acid and ulcer index and a significant increase in the pH values, as compared to the control values. [Table - 2]

Effects of combined treatments

Combined treatment consisting of famotidine (1 mg/kg) with MEPA (200 mg/kg) produced a significant (P <0.01) decrease in the volume of gastric secretion, free acid, total acid and ulcer index with a significant increase in the pH values, as compared to the control values as well as to the groups that received either famotidine or MEPA alone. [Table-3]

Combination of omeprazole (1 mg/kg) and MEPA (200 mg/kg) also produced a significant (P <0.01) decrease in the values of all the parameters, with a significant increase in the pH values, as compared to the control values as well as to the groups that received either omeprazole or MEPA alone. [Table - 3]

DISCUSSION

The etiology of peptic ulcer is unknown in most of the cases, yet it is generally accepted that it results from an imbalance between aggressive factors and the maintenance of mucosal integrity through the endogenous defense mechanisms.[11] To regain the balance, different therapeutic agents are used to inhibit the gastric acid secretion or to boost the mucosal defense mechanisms by increasing mucus

production, stabilizing the surface epithelial cells or interfering with the prostaglandin synthesis.[12] The causes of gastric ulcer after pyloric ligation are believed to be due to stress-induced increase in gastric hydrochloric acid secretion and/or stasis of acid. According to Shay *et al.*, 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 acid.

Famotidine, a competitive antagonist of H_2 - receptor, is capable of reducing over 90% of basal and nocturnal secretion of gastric acid and that stimulated by food, histamine, gastrin, cholinomimetic drugs and vagal stimulation[13] . Famotidine reduces acidity and volume of gastric secretion by blocking the effect of histamine. It can also reduce the gastrin and the vagus nerve-mediated secretion to some extent. Famotidine exerts its antisecretory effect by inhibiting the histamine-induced cyclic-AMP-dependent pathway.[14] The proton pump inhibitor omeprazole produces small and inconsistent changes in the volume of gastric secretion and in the secretion of pepsin, but it does not affect gastric motility. It irreversibly inhibits the gastric acid (proton) pump which is the final common pathway for acid secretion in response to all varieties of stimuli. It produces virtual anacidity *in vivo*.

In the present study, therapeutically equivalent doses of MEPA (200 mg and 400mg/kg) produced significant decrease in the volume, free acidity and total acidity of the gastric secretion along with a significant protective effect against gastric ulceration induced by pylorus ligation. A lower dose of 200 mg/kg did not produce any significant alteration in the parameters studied.

Gastric acid secretion is under vagal control and overactivity of vagus also contributes to ulcer formation.[9] Vagal stimulation increases acetylcholine that acts directly on the muscarinic receptors on parietal cells and secretes hydrochloric acid through a calcium-dependent pathway.[16] Mandal *et al.* The gastric antisecretory effect of MEPA may be due to a may partly be due to inhibition of calcium-dependent pathway.

A role for reactive oxygen metabolites, free radicals and nitric oxide has been suggested in the pathogenesis of gastric ulcer. Recently, it I was proved that MEPA has antioxidant activity [15-18]. Thus, it is tempting to suggest that the MEPA-induced protective effects in pylorus-ligated gastric ulcers could be in part also mediated through either a decrease in free-radical generation or an increase in nitric oxide production. Further study is necessary to identify the role of MEPA on the above factors. In the present study, low doses of either famotidine (1 mg/kg), omeprazole (1 mg/kg) or MEPA (200 mg/kg) did not produce any antiulcer effect. Higher doses of MEPA (400 mg/kg) produced a significant protective effect against experimental gastric ulceration in rats. However, a combination of low dose (200 mg/kg) of MEPA with low dose (1 mg/kg) of famotidine produced a significant antiulcer effect. Similarly, a low dose of MEPA (200 mg/kg) combined with a low dose of omeprazole (1 mg/kg) also produced significant antiulcer activity [19, 20].

In histopathological study there is no significant change in famodine and omeprazole treatment in low dose (1mg/kg). But in combination with MEPA in low dose (200mg/kg), it was showed the significant ulcer recovery (Figure 2).

Hence, simultaneous administration of low doses of MEPA with either famotidine or omeprazole may produce significant antiulcer effects, as reflected by the reduction of volume of gastric secretion, acidity and ulcer index.

CONCLUSION

From the present investigation, it may be concluded that MEPA produced a significant protective effect against gastric ulceration and also produced significant antiulcer effects when used in

combination with famotidine and omeprazole. It is to be studied whether doses of famotidine and omeprazole could be reduced for the management of peptic ulcer in patients who are on MEPA therapy.

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Table.1: Effect of famotidine and omeprazole on volume of gastric secretion, pH, free acid total acid and ulcer index in pylrus ligated rate

Group	valume of Gastric secretion ml	PH	Free acid(mEq/L)	Total acid(mEq/L)	Ulcer Index
Control	4.48±0.11	2.53±.11	55.00±1.06	146.63±1.20	0.342±0.015
Famotidine 1mg/kg 4mg/kg	 4.35±0.12 2.25±.08*	 2.42±.20 4.25±0.12*	 64.50±1.48 33.50±1.02*	 154.83±1.01 85.12±1.23*	 0.449±1.006 0.22±.003*
Omeprazole 1mg/kg 4mg/kg	 4.22±0.11 1.53±0.05*	 2.45±0.10 5.22±0.06*	 74.33±1.05 22.14±1.05*	 161.57±1.05 89.56±1.06*	 0.428±.002 0.165±.002*

The data are expressed as mean ±S.E.M. Significant differences in each group versus the control were as follows * P < 0.05. ** P < 0.01.

Table.2: Effect of MEPA on volume of gastric secreation, pH, free acid, total acid and ulcer index in pylorus-ligated rats.

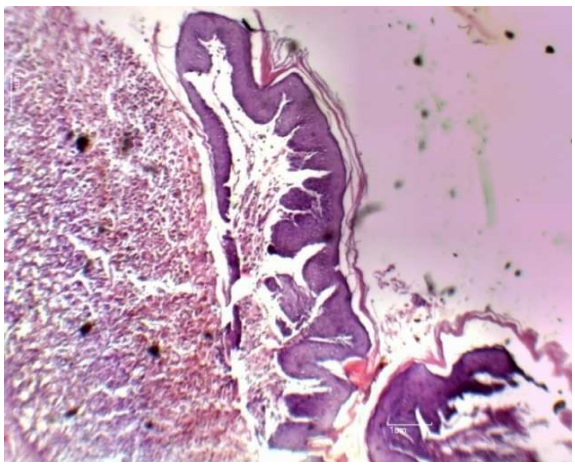
Group	volume of Gastric secretion ml	PH	Free acid(mEq/L)	Total acid(mEq/L)	Ulcer Index
Control	4.48±0.11	2.53±.11	55.00±1.06	156.63±1.20	0.342±0.015
MEPA **200mg/kg** **400mg/kg**	 4.33±0.11 4.22±0.12*	 2.22±0.11 4.12±0.13*	 79.20±0.48 46.20±1.08*	 148.20±1.150 143.65±1.03	 0.528±.004 0.320±.008*

The data are expressed as mean ±S.E.M. Significant differences in each group versus the control were as follows * P < 0.05. ** P < 0.01.

Table.3: Effects of combined treatments on volume of gastric secreation, pH, free acid, total acid and ulcer index in pylorus-ligataed rats.

Group	valume of Gastric secretion (ml)	PH	Free acid(mEq/L)	Total acid(mEq/L)	Ulcer Index
Control	4.48±0.11	2.53±.11	55.00±1.06	146.63±1.20	0.342±0.015
MEPA 200mg/kg 400mg/kg	 4.33±0.11 4.22±0.12*	 2.22±0.11 4.12±0.13*	 79.20±0.48 46.20±1.08*	 148.20±1.150 143.65±1.03	 0.528±.004 0.320±.008*
Famotidine 1mg/kg	4.35±0.12	2.42±0.20	64.50±1.48	154.83±1.0	0.449±1.006
Omeprazole 1mg/kg	4.22±0.11	2.45±0.10	74.33±1.05	161.57±1.05	0.428±.002
MEPA(200mg/kg)+ Famotidine (1mg/kg)	1.22±0.06**	4.02±0.13**	27.13±1.14**	85.02±1.00**	0.235±.006**
MEPA(200mg/kg)+ Omeprazole (1mg/kg)	1.28±0.03**	4.23±0.13**	23.07±1.34**	85.53±1.21**	0.154±0.005**

The data are expressed as mean ±S.E.M. Significant differences in each group versus the control were as follows * P < 0.05. ** P < 0.01.



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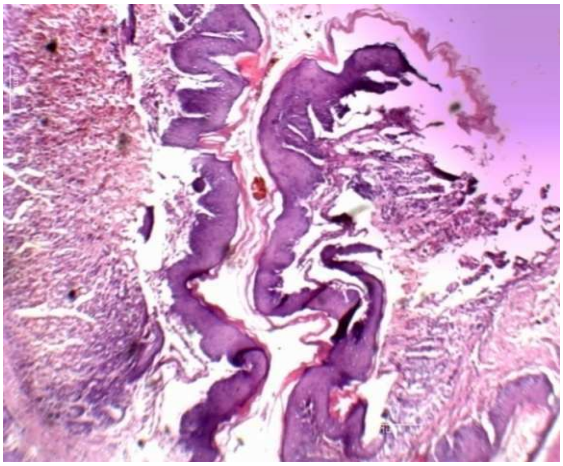

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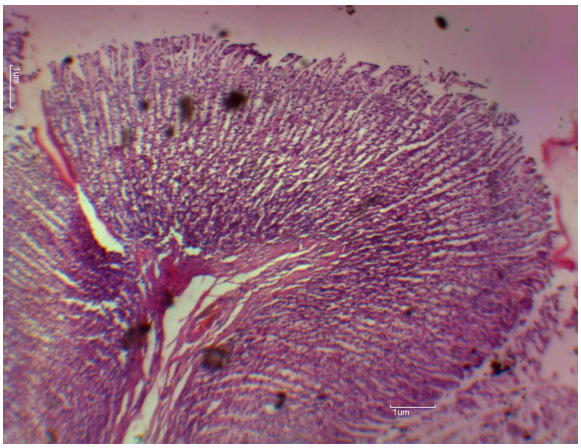

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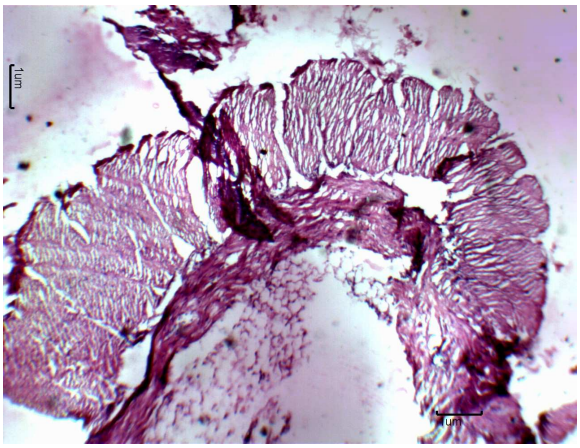

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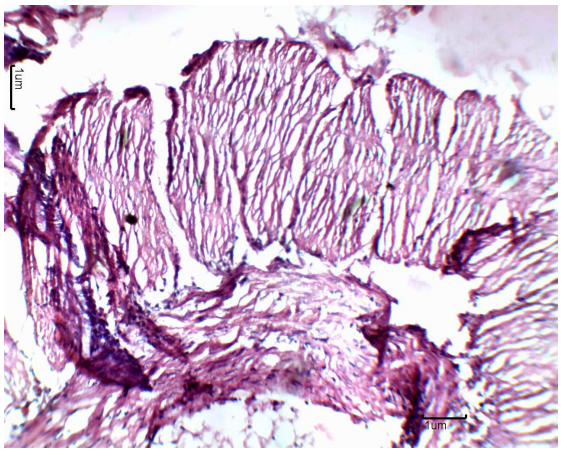

(e)

Fig.1 (A-B): Represents the stomach histology.

(A) Stomach histology of control dose.
(B) Stomach histology of low dose famotidine (1mg/kg) where no significant recovery of ulcer.
(C) Stomach histology of low dose omeprazole (1mg/kg) where no significant recovery of ulcer.
(D) Stomach histology of low dose famotidine (1mg/kg) and low dose of MEPA (200mg/kg) where significant recovery of ulcer showed.
(E) Stomach histology of low dose famotidine (1mg/kg) and low dose of MEPA (200mg/kg) where significant recovery of ulcer showed

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