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Research Article

ANTIULCER ACTIVITY OF *PREMNA SERRATIFOLIA* AGAINST ASPIRIN INDUCED GASTRIC ULCER MODEL

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ABSTRACT

The present study was carried out to investigate antiulcer activity of methanolic bark extract of *Premna serratifolia* against aspirin induced gastric ulcer models of male wistar rats. In aspirin induced pylorus ligation model, various parameters were studied viz. volume and pH of gastric juice, total acidity, free acidity, ulcer score, ulcer index and percentage protection was determined. Ulcer score, ulcer index and percentage inhibition of ulceration was determined for ulcer model. Ranitidine (50mg/kg p.o.) was used as the standard drug. Pretreatment with the extracts (200 & 400mg/kg p.o.) showed significant protection against ulcer model. In aspirin induced pylorus ligated model, the methanolic bark extract showed significant decrease in the volume of gastric juice, free and total acidity, ulcer score, ulcer index and increase in pH of gastric juice as compared to the toxicant control group. In conclusion, *Premna serratifolia* possess significant anti-ulcer and cytoprotective effect.

Keywords: *Premna serratifolia,* anti-ulcer, Pylorus ligation, Ulcer index.

INTRODUCTION

Peptic ulcer occurs due to imbalance between aggressive (acid, pepsin) and defensive (mucus gastric mucosal barrier) factors of gastric mucosa. Local mechanisms implicated in mucosal defense are mucus-alkaline secretion, mucosal hydrophobicity, rapid epithelial cell renewal and rich mucosal blood flow[1]. Prostaglandins E2 and I2 are the predominant prostaglandins synthesized by the gastric mucosa and are known to inhibit the secretion of gastric acid and stimulate the secretion of mucus and bicarbonate[2]. The treatment of peptic ulcer is directed against either reduction of aggressive factors or enhancement of mucosal defense of stomach and duodenum with cytoprotective agents. Ulcer is defined as the erosion in the lining of the stomach or duodenum and is caused by the disruptions of the gastric mucosal defense and repair system[3]. Ulcer in the stomach is called gastric ulcer and in the duodenum is called duodenal ulcer and together peptic ulcer. In clinical practice, peptic ulcer is one of the most prevalent gastrointestinal disorders, commonly occurs in developed countries. Treatments available for ulcer is generally non-specific and is usually aimed at reducing the production of gastric acid and re-enforcing gastric mucosal protection such as regular food, adequate rest and avoidance of ulcerogenic agents such as coffee, alcohol and tobacco. The drugs used in the treatment of ulcer include receptor blockers, proton pump inhibitors, drugs affecting the mucosal barrier and act on the central nervous system[4]. Even though a range of drugs are available for the treatment of ulcer, many of these do not fulfill all the requirements and have side effects[5,6]. Recently, there has been much interest in natural medicines derived from the traditional knowledge of plant pharmacological properties. Large number of medicinal plants and dietary nutrients has been shown to possess gastro-protective activity[7-11]. *Premna serratifolia* Linn, (Verbenaceae) is an important plant belonging to the family Verbenaceae and is one of the most widespread large shrubs in the forests of India, usually occurring in deciduous forests. The whole plant possesses medicinal properties, useful in the treatment of cardiovascular diseases, skin diseases, inflammatory diseases, arthritis, gonorrhea, rheumatism, anorexia and jaundice. It is an important Ayurvedic medicinal herb and its synonym is *Premna integrifolia*. It is popularly known as"Munney" in Tamil and "Agnimantha" in Ayurvedic system of medicine. Root forms an ingredient in well known Ayurvedic formulation "Dasamula" which is used for variety of affections. It is widespread throughout Micronesia and much of the tropical Pacific and tropical Asia. It is common along the Indian and Andaman coasts. Infusion of the leaves is administered with pepper in cold and fever. Leaves are used to cure "weakness of limbs" and the leaves and leaf sap were used to alleviate headache[12]. *Premna serratifolia* Linn has cardiotonic[13], anti-coagulant[14], anti-inflammatory[15], antihyperglycaemic[16], anti-parasitic[17], antioxidant[18] and antimicrobial[19] properties. Most of the plant parts of *Premna serratifolia* Linn have been used in the traditional system of medicine in India to treat various infectious diseases. In view of these the present study has been undertaken to evaluate in detail the antiulcer effect of the methanolic bark extract of *Premna serratifolia* in aspirin induced gastric ulcer model.

MATERIALS AND METHODS

Plant Material

The dried bark of *Premna serratifolia* was collected from the local fields of Thrissur, Kerala, India.

Preparation of Extract

Dried bark of *Premna serratifolia* were powdered and subjected to batch-wise extraction in soxhlet apparatus using methanol as solvent. The extracts were then concentrated to dryness on water bath and stored in refrigerator until use.

Preliminary Phytochemical Testing of Extracts

The extract obtained were subjected to preliminary phytochemical investigation this showed the presence of alkaloids, flavonoids, phenols, steroids and glycosides.

Experimental Design

The animals are divided into four groups of six rats in each group.

Group I: Serves as negative control.

Group II: Serves as positive control was given std. drug, (ranitidine 50mg/kg) orally.

Group III: Serves as treated ulcer group was given standard diet and 200mg/kg bw (plant extract), orally.

Group IV: Serves as treated ulcer group was given standard diet and 400mg/kg bw (plant extract), orally.

Aspirin Induced Pylorus Ligated (Shay) Rats Method

Adult Albino rats weighing 180-250gm, approximately of the same age and fed on standard chow diet were used. They were administered with aspirin (200mg/kg) through oral route without fasting, after 30minutes of Extract was administered in different doses (50,100 and 200mg/kg bw, orally) for five days. At the end of the fifth day the animals were kept fasted for 14hrs with water ad libtium, animals were treated with extract 30minutes before ligation and the pylorus was ligated under light ether anesthesia, care being taken not because bleeding or to occlude blood vessels. After 30minutes again the animal is treated with aspirin. The animals were sacrificed 6hours after pylorus ligation. The stomachs were removed, contents collected, volume measured and centrifuged. One milliliter of supernatant was titrated against 0.01N NaOH to determine the acidity using phenolphthalein as indicator and total acid output calculated.

Determination of Acid Secretary Parameters

Determination of Total Acidity

Procedure

10ml of gastric juice specimen was transferred in a porcelain evaporating dish. 1-2 drops of Topfer's reagent was added. A colour change was observed, a bright red colour appears if free hydrochloric acid is present. 1-2 drops of phenolphthalein was added to the gastric juice with Topfer's reagent. Titrated with 0.01 N NaOH from a burette, mixing was done after each addition until the last trace of red colour disappeared and was replaced by a canary yellow colour. The numbers of milliliters of NaOH used was read from the burette. This represents the amount of free hydrochloric acid. The titration was continued until the red colour of phenolphthalein appeared (deep pink), titrated to the point at which the further addition of alkali did not deepen the colour. Reading was taken (ml NaOH) for total acidity.

Total acidity (mEq/l) = ml of 0.01 N NaOH x100xNormality/0.1

Ulcer Index

Procedure

The stomachs were removed and fixed on a cork plate and the number and severity of ulcers was registered with a stereo-microscope using the following scores.

Severity score

0 = Normal coloured stomach 0.5 = Red colouration

1 = Spot ulcer 1.5 = Hemorrhagic streaks

2 = Ulcers ≥ 3 but ≤5 3 = ulcers > 5

Ulcer index was calculated as:

Ulcer index (UI) = [10*ulcerated area (mm2)/ total stomach area (mm2)]

Acid Volume

Procedure

The stomachs were removed and the contents were drained into a graduated centrifuge tube through a small nick along the greater curvature. The tubes were centrifuged at 3000rpm for 10min and the centrifuged samples were decanted and measured.

Ulcer Protective

% Protective = Control mean ulcer index – Test mean ulcer index / Control mean ulcer index × 100

Statistical Analysis

Values are expressed as Mean ± SEM, One-way ANOVA followed by Dunnett's Test ***p<0.001 when compared with negative control (group I) with standard (group II), treatment 200 &400mg/kg (group 3&4)

Table 1: Effect of *Premna serratifolia* bark extract on free acidity in aspirin induced gastric ulcers in rats

Group	Free acidity mEq/l
Negative Control(aspirin+PL)	92.45±0.856
Aspirin+PL +Standard(50mg/kg)	22.00 ±1.528 a***
Aspirin+PL +Extract(200 mg/kg)	51.23 ±2.856b***
Aspirin+PL +Extract(400mg/kg)	42.32±1.887c***

Values are expressed as mean ±SD (n=6).Group 1: Negative Control, Group 2: Standard, Group 3: Low dose, Group 4: High dose. [a] Compared with group1 and group2; [b] Compared with group2 and group3; [c] Compared with group2 and group4;** *p<0.001 (One-way ANOVA followed by Fisher's test). (Daniel 2006)

Table 2: Effect of *Premna serratifolia* bark extract on total acidity in aspirin induced gastric ulcers in rats

Group	Total acidity mEq/l
Negative Control(aspirin+PL)	110.33 ± 2.856
Aspirin+PL +Standard(50mg/kg)	28.00±2.528a***
Aspirin+PL +Extract(200 mg/kg)	53.00 ±2.528b***
Aspirin+PL +Extract(400mg/kg)	46.03± 1.887c***

Values are expressed as mean ±SD (n=6).Group 1: Negative Control, Group 2: Standard, Group 3: Low dose, Group 4: High dose. [a] Compared with group1 and group2; [b] Compared with group2 and group3; [c] Compared with group2 and group4; ** *p<0.001 (One-way ANOVA followed by Fisher's test). (Daniel 2006)

Table 3: Effect of *Premna serratifolia* bark extract on percentage ulcer protection in aspirin induced gastric ulcers in rats

Group	Percentage Ulcer Protection (%)
Negative Control(aspirin+PL)	0
Aspirin+PL +Standard(50mg/kg)	86.13
Aspirin+PL +Extract(200 mg/kg)	51.70
Aspirin+PL +Extract(400mg/kg)	59.60

Values are expressed as mean ±SD (n=6).Group 1: Negative Control, Group 2: Standard, Group 3: Low dose, Group 4: High dose. [a] Compared with group1 and group2; [b] Compared with group2 and group3; [c] Compared with group2 and group4;. ** *p<0.001 (One-way ANOVA followed by Fisher's test). (Daniel 2006)

Table 4: Effect of *Premna serratifolia* bark extract on gastric volume in aspirin induced gastric ulcers in rats

Group	Gastric volume
Negative Control(aspirin+PL)	5.580±1.45
Aspirin+PL +Standard(50mg/kg)	1.640±0.213a***
Aspirin+PL +Extract(200 mg/kg)	3.800±2.515b***
Aspirin+PL +Extract400mg/kg)	2.510±0.254c***

Values are expressed as mean ± SD (n=6).Group 1: Negative Control, Group 2: Standard, Group 3: Low dose, Group 4: High dose. [a] Compared with group1 and group2; [b] Compared with group2 and group3; [c] Compared with group2 and group4; ** *p<0.001 (One-way ANOVA followed by Fisher's test). (Daniel 2006)

Table 5: Effect of *Premna serratifolia* bark extract on ulcer index in aspirin induced gastric ulcers in rats

Group	Ulcer index
Negative Control(aspirin+PL)	14.06±0.569
Aspirin+PL +Standard(50mg/kg)	1.95±2.154
Aspirin+PL +Extract(200 mg/kg)	6.79±0.365
Aspirin+PL +Extract(400mg/kg)	5.68±1.854

Values are expressed as mean ± SD (n=6).Group 1: Negative Control, Group 2: Standard, Group 3: Low dose, Group 4: High dose. [a] Compared with group1 and group2; [b] Compared with group2 and group3; [c] Compared with group2 and group4;** *p<0.001 (One-way ANOVA followed by Fisher's test). (Daniel 2006)

RESULTS

Phytochemical analysis

The phytochemical analysis of methanolic bark extract of *Premna serratifolia* showed the presence of alkaloids, flavonoids, phenols, steroids and glycosides.

Antiulcer Study

The antiulcer activity of *Premna serratifolia* bark extract in aspirin induced gastric ulcer model is illustrated in Table 1-5. The anti-ulcer activity of *Premna serratifolia* bark extract in aspirin induced gastric ulcer model is evident from its significant reduction in gastric volume, free acidity, total acidity and ulcer index. Because the plants extract treated animals significantly inhibited the formation of aspirin induced gastric ulcer in the stomach and also decreased both acid concentration and gastric volume. The increase in the gastric volume of the untreated Aspirin+PL group is undoubtedly due to increased production of HCL as it is evident from the total acidity of the gastric juice. At the same time methanolic bark extract of *Premna serratifolia* (200mg/Kg) and methanolic bark extract of *Premna serratifolia* (400mg/Kg) treated groups did not produce any significant changes in the biochemical parameters and they preserved the normal architecture of the stomach mucosa by significantly reducing the gastric volume and ulcer index as compared to PL control animals. This further establishes the fact that the extracts have ulcer protective in nature.

DISCUSSION

Ulcers are defined histologically as a breach in the mucosa of the alimentary tract that extends through the muscularis mucosa into the submucosa or deeper. Although they may occur anywhere in the alimentary tract, none are as prevalent as the peptic ulcers that occur in the duodenum and stomach. Peptic ulcers are relapsing lesions that are most seen in middle-aged to older adults but they may first become evident in young adult life[20,21]. Peptic ulcer and gastritis have been associated with multipathogenic factors and could be due to disturbances in natural balances between the aggressive factors (e.g. of acid, bicarbonate, pepsin) and maintenance of the mucosal integrity through the endogenous defense mechanism (e.g. of defensive mechanisms of mucus, mucosal turnover and blood supply (mucosal barrier). Despite the availability of many pharmaceutical products for the treatment of gastric ulcers in the market as mentioned above, their successes were limited by presence of several adverse effects (e.g. anaphylaxis reactions, gynecomastia, hematopoietic changes, thrombocytopenia, acute interstitial nephritis, nephrotoxicity and hepatotoxicity). Aspirin causes mucosal damage by interfering with prostaglandin synthesis, increasing acid secretion, decreasing mucin activity and back diffusion of H+ ions. Pylorus ligation induced ulcers are due to auto digestion of the gastric mucosa and breakdown of the gastric mucosal barrier[22]. Further the antiulcer assay of *Premna serratifolia* showed a decrease in the gastric acid secretion and thus decreasing the prevalence for causing ulcer. These results confirmed the anti-secretory potency as well as acid neutralizing effect of the plant extracts. However, further studies are required to establish its exact mode and the active principles involved in its anti-ulcer effect.

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