

Gastroprotective and antisecretory properties of methanolic extract of *Trianthema portulacastrum*. L in aspirin & pyloric ligature induced gastric ulcer in rats

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ABSTRACT

Objective: The present study was carried out to investigate antiulcer activity of methanolic extract of whole plant of *Trianthema portulacastrum* L. belonging to the family of Aizoaceae in pylorus ligated and aspirin induced gastric ulcer in rats. **Methods:** Preliminary methanol extract of *Trianthema portulacastrum* L. (METP) was subjected to the acute oral toxicity study according to the OECD guideline no. 425. Based on which, two dose levels i.e. 250 and 500 mg/kg were selected for the further study. In both models, we assessed ulcer index, gastric juice volume, gastric pH and total acidity. Ranitidine (50 mg/kg) was used as the positive control. **Results:** In present study, METP showed significantly decrease in ulcer index in both ulcer models in a dose dependent manner. METP showed a dose dependent significant decrease in gastric juice and total acidity in both the models. A dose dependent increase in gastric pH and total adherent gastric mucus was also produced in METP treated groups. **Conclusion:** The present study suggested that the antiulcer activity of METP on pylorus ligation induced gastric ulcer can due to anti secretory activity. The mechanism of protection against aspirin induced gastric ulcer can be attributed to 5- lipoxygenase pathway.

Key words: Aspirin, Gastric ulcer, Pylorus ligated, *Trianthema portulacastrum*, Ulcer index.

INTRODUCTION

Gastric ulcer, which affects thousands of people, is becoming one of the most important diseases of the digestive system and a medical-social problem of global economic importance due to its higher and higher morbidity and mortality.^{1,2} It is an imbalance between damaging factors within the lumen and protective mechanism within the mucosal membrane. The excess gastric acid formation by prostaglandin includes both increases in mucosal resistance as well as decrease in aggressive factors mainly acid and pepsin.³ Stress, smoking, nutritional deficiencies, ingestion of nonsteroidal anti-inflammatory drugs, hereditary predisposition and infection by Helicobacter pylori are all factors that can increase the incidence of gastric ulcer.⁴ A lot of drugs are available for the treatment of gastric ulcer such as H₂ receptor antagonist, proton pump inhibitors, anticholinergics and antacids.⁵ Adverse drug reactions from these drugs demands for the use of complementary herbal medicine.⁶ Although many drugs have been synthesized and established for gastric ulcer treatment, the search

for agents having powerful antiulcer activity with possible natural biological occurrence and predictably having no side effects is worthwhile. The use of herbal medicine has been reported in the treatment of gastric ulcer disease (GUD) condition.⁷

Trianthema portulacastrum (TP) is a plant of the family Aizoaceae, found almost throughout India as a weed in cultivated and waste-lands. The plant is bitter, hot, alexiteric, analgesic, stomachic, laxative, and serves as alterative cures for bronchitis, heart disease, blood anaemia, inflammation and piles ascites.⁸ The root is applied to the eye to treat corneal ulcers, itching, dimness of sight, and night blindness.⁹ The plant has a remarkable protection against the induction of hepatotoxicity.¹⁰ It was also reported that the hypoglycemic and hypolipidemic activity of methanolic extract of *Trianthema portulacastrum* (METP) in alloxan-induced diabetic rats.¹¹ Despite the herbal use of TP as a gastro intestinal disorders, to date existing literature fails to evidence its anti ulcer activity. Therefore, the aim of the present study was to evaluate the anti ulcer effect of methanolic extract of *Trianthema portulacastrum* on pyloric ligated and aspirin induced gastric ulcer in rats

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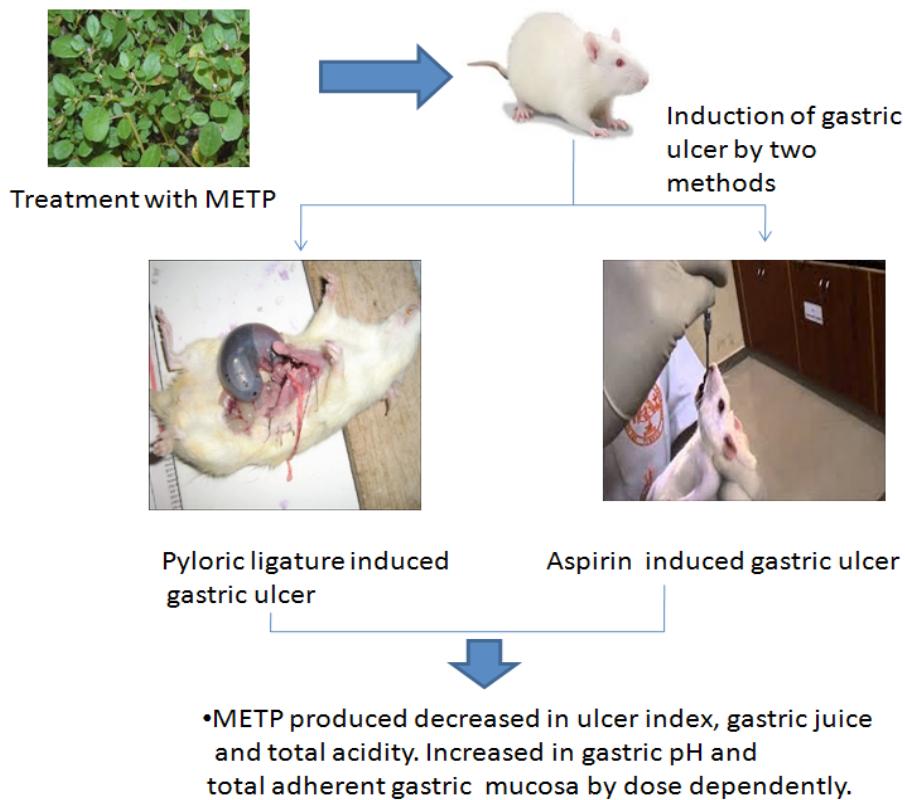
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MATERIALS AND METHODS

Plant material

The whole plants of *Trianthema portulacastrum* L. were collected from Chennai, Tamilnadu during September 2013. It was identified and



Conclusion: Antiulcer activity of METP on pylorus ligation induced gastric ulcer can due to anti secretory activity. The mechanism of protection against aspirin induced gastric ulcer can be attributed to 5- lipoxygenase pathway.

Graphical Abstract

authenticated by Prof. P.Jayaraman Ph.D., Director-Plant Anatomy Research Centre (PARC) Tambaram. The fresh plants were shade dried, powdered and stored in air tight container for further use.

Preparation of plant extract

The shade dried, coarsely powdered plants (500gm) were successively extracted with petroleum ether (60-80 °c) for 8 hr to remove fatty matter. The defatted marc was then subjected to soxhlet extraction with 95% methanol to obtain methanolic extract. The methanolic extract were evaporated under reduced pressure at low temperature (30°C) to dryness and yield extracts of *Trianthema portulacastrum*, stored in an airtight container in refrigerator for further experimental studies.

Phytochemical Screening

Methanolic extract of *Trianthema portulacastrum* (METP), were subjected to preliminary phytochemical screening for the detection of various plants constituents.¹²

Procurement and Maintenance of Experimental Animals

Adult Wistar rats (180-200 g) were procured from C.L Baid Metha College of Pharmacy, Thorapakkam, and Chennai and divided into 9 groups of 6 animals each. The rats were housed in colony cages at an ambient temperature of 25°C ± 2°C and 40-65% RH with a 12:12 h L:D cycle. The animals had free access to standard pellet chow and drinking water. The study was approved by institutional animal ethical committee (IAEC) and work was carried out as per CPCSEA guidelines, India.

Chemicals

Rantidine and Aspirin (Madras pharmaceuticals Pvt Ltd. Chennai). All other chemicals and reagents unless specified were of analytical grade.

Experimental design

Phytochemical analysis

The phytochemical analysis of the METP was carried out using standard phytochemical procedures and tests

Acute toxicity study

Acute toxicity studies were performed according to organization for economic co-operation and development (OECD) guidelines. Animals were divided in groups (n=5). The animals were fasted for 4 h. with free access to water only. The METP extracts was administered orally in doses of 2500 and 5000 mg/kg to different groups of rats and observed over 14 days for mortality and physical/behavioral change.¹³

Assessment of Anti-Ulcer Activity

Pyloric ligation induced gastric ulceration¹⁴

Albino rats of either sex were divided into five groups of six animals each. Animals were fasted for 24 h before the study, but had free access to water. Group 1 animals were received with normal saline. Group 2 animals were pyloric ligated alone, whereas animals of group 3 and 4 received with METP at the dose of 250 and 500 mg/kg, (p.o.) and group 5 animals received with ranitidine 50 mg/

kg, (p.o.) along with pyloric ligation and were served as positive control. After 1 hr of treatment with METP or ranitidine, the rats were anaesthetized using chloroform. Their abdomen was opened by a small midline incision below the xiphoid process. The stomach was closed up carefully by interrupted sutures. The animals were allowed to recovery and stabilization and were deprived of water during postoperative period. The animals were then sacrificed by an over dose of chloroform inhalation after 4h of pyloric ligation to observe gastric lesions.

Aspirin induced gastric lesions¹⁵

Group 6 animals received with aspirin (200 mg/kg) alone to induce gastric ulcer, while animals of group 7 and 8 received with METP (250 and 500 mg/kg) and group 9 animals were received with ranitidine 50 mg/kg, (p.o.) along with aspirin. Animals of the test group received plant extract orally for 10 days. From day 8 the animals received saline/METP two hours prior to the administration of aspirin. Overnight fasted animals were sacrificed with cervical dislocation one hour after last dose of aspirin. The stomach was incised along the greater curvature and examined.

Statistical analysis

Results are represented as mean \pm SEM. Statistical difference between the means of the various groups was analyzed using one way ANOVA followed by Tukey's Multiple Comparison test. Data were considered statistically significant at $p<0.05$.

RESULTS

Phytochemical analysis

Preliminary phytochemical analysis of the methanolic extract of *Trianthema portulacastrum* (METP) revealed the presence of alkaloids, flavonoids, phenolics, steroids, tannins and saponins.

Acute toxicity studies

Acute toxicity was carried out by up down regulation method. It was found that METP were safe at limit dose 2500 mg/kg and 5000 mg/kg with no mortality in studied subjects. 1/10th of these doses i.e. 250 mg/kg and 500 mg/kg were used in the subsequent study respectively.

Effect of METP on pylorus ligated rats

METP showed a dose dependent protection against pyloric ligation induced gastric ulcer in rats. Pylorus ligation in ulcerated group had significantly ($p<0.001$) increased the ulcer index when compared with control rats. METP (250 and 500 mg/kg) and ranitidine treated rats showed significantly ($p<0.001$) increased the ulcer index when compared with control rats. However, the ulcer index showed dose dependently decreased in the METP treated rats when compared with pyloric ligated rats. In pyloric ligated treated rats significantly ($p<0.001$ and $p<0.001$) increased the gastric volume, total acidity and significantly ($p<0.001$) decreased the gastric pH when compared with control rats. METP produced a dose dependent reduction in gastric juice volume and total acidity, but maximum reduction in these parameters was produced by ranitidine treated group. Gastric pH was also found to be significantly ($p<0.001$) decreased in all drug treated groups when compared with control group. METP (500 mg/kg) and ranitidine treated rats significantly ($p<0.01$ and $p<0.001$) increased the gastric pH as comparison with pyloric ligated group (Table 1).

Effect of METP on aspirin induced gastric ulcer

In the present study, METP was evaluated for its anti ulcer activity against aspirin induced gastric ulceration in rats. All drug treated group showed significantly ($p<0.001$) increased the ulcer index as compared to normal saline treated group. METP (250 and 500 mg/kg) and ranitidine treated rats showed significantly ($p<0.05$, $p<0.001$)

Table 1: Effect of METP on pyloric ligature induced gastric ulcer in rats

Treatment	Ulcer Index (% protection)	Gastric Volume (ml/100 g body weight)	Gastric pH	Total acidity
Control	0.60 \pm 0.05	1.26 \pm 0.48	4.65 \pm 1.26	28.41 \pm 0.68
Pyloric ligated rats	3.60 \pm 0.96 ^c	5.08 \pm 2.08 ^c	1.96 \pm 1.38 ^c	64.48 \pm 0.84 ^c
METP (250 mg/kg) + Aspirin (200 mg/kg)	3.48 \pm 1.08 ^c	3.96 \pm 0.46 ^{c,z}	2.66 \pm 2.18 ^c	39.48 \pm 0.46 ^{b,z}
METP (500 mg/kg) + Aspirin (200 mg/kg)	3.20 \pm 0.14 ^c	3.08 \pm 1.48 ^{c,z}	2.78 \pm 1.48 ^{b,y}	37.43 \pm 0.96 ^{b,z}
Ranitidine (50 mg/kg) + Aspirin (200 mg/kg)	1.48 \pm 0.10 ^{b,z}	2.16 \pm 1.46 ^{c,z}	3.68 \pm 0.28 ^{b,z}	29.39 \pm 1.08 ^z

Values are expressed as mean \pm SEM. Significance with mean Tukey's multiple comparison test followed by one way ANOVA is indicated symbol denote the significance level. ^a $p<0.05$, ^b $p<0.01$ and ^c $p<0.001$ when compared with control group ^x $p<0.05$, ^y $p<0.01$ and ^z $p<0.001$ when compared with pyloric ligated rats.

Table 2: Effect of METP on aspirin induced gastric ulcer in rats

Treatment	Ulcer Index (% protection)	Gastric Volume (ml/100 g body weight)	Gastric pH	Total acidity
Control	0.60 \pm 0.05	1.26 \pm 0.48	4.65 \pm 1.26	28.41 \pm 0.68
Aspirin (200 mg/kg)	4.83 \pm 1.16 ^c	5.48 \pm 1.35 ^c	2.16 \pm 0.45 ^c	78.13 \pm 1.72 ^c
METP (250 mg/kg) + Aspirin (200 mg/kg)	3.16 \pm 2.48 ^{c,x}	4.16 \pm 2.26 ^{c,y}	2.92 \pm 1.16 ^c	45.18 \pm 2.16 ^{c,z}
METP (500 mg/kg) + Aspirin (200 mg/kg)	2.08 \pm 1.28 ^{c,z}	4.02 \pm 0.72 ^{c,y}	3.16 \pm 0.95 ^{b,y}	39.83 \pm 1.86 ^{c,z}
Ranitidine (50 mg/kg) + Aspirin (200 mg/kg)	1.68 \pm 1.10 ^{c,z}	3.76 \pm 0.06 ^{c,z}	3.45 \pm 1.32 ^{b,z}	30.46 \pm 1.68 ^{c,z}

Values are expressed as mean \pm SEM. Significance with mean Tukey's multiple comparison test followed by one way ANOVA is indicated symbol denote the significance level. ^a $p<0.05$, ^b $p<0.01$ and ^c $p<0.001$ when compared with control group ^x $p<0.05$, ^y $p<0.01$ and ^z $p<0.001$ when compared with aspirin treated rats.

and $p<0.001$) increased the ulcer index when compared with aspirin alone treated group. Maximum protection was observed in ranitidine treated group when compared with aspirin alone treated group. The volume of gastric secretion and total acidity was significantly ($p<0.001$) in all drug treated groups as compared to normal saline treated group. METP (250 and 500 mg/kg) and ranitidine treated rats showed significantly ($p<0.001$) reduction in gastric secretion and total acidity as compared with aspirin alone treated group. Significant reduction in gastric pH in all drug treated groups when compared with control rats. METP (500 mg/kg) and ranitidine treated rats showed significantly ($p<0.01$ and $p<0.001$) increased the gastric pH when compared to aspirin alone treated group (Table 2).

DISCUSSION

Gastric ulcer may develop when the normal defense and repair mechanisms of the lining of the stomach or duodenum are weakened, making the lining more likely to be damaged by gastric acid. Pathophysiology of gastric ulcer involves an imbalance between offensive or injurious (acid, pepsin and *Helicobacter pylori*) and defensive mucosal factors (mucin, prostaglandin, bicarbonate, nitric oxide and growth factors). Gastric acid plays a major role in the pathogenesis of gastric and duodenal ulcers. Lots of allopathic drugs are available in the market to treat for gastric ulcer such as antacids, proton pump inhibitors, anticholinergics, histamine H₂ receptor antagonist are used. These drugs can produce several adverse effects such as hepatotoxicity,¹⁶ nephrotoxicity and thrombocytopenia etc., Therefore, herbal medicine are much better alternatives for treatment of gastric ulcer with lesser adverse effects.

In the preliminary phytochemical evaluation performed in the present study demonstrated that the METP contains alkaloids, flavonoids, phenolics, steroids, tannins and saponins. Previous reports supports for our present investigation, flavonoids are implicated in the protection of the gastric mucosa from necrotizing substances and flavonoids are highly useful in the therapy of acute and chronic gastric ulceration.^{17,18}

The present study discussed that *Trianthema portulacastrum*.L exhibits both gastroprotective and ulcer healing properties. In both aspirin and pylorus ligated induced gastric ulcer in rat models, METP reduced the ulcer index thus showing the anti ulcerogenic activity.

Highlights of Paper

- *Trianthema portulacastrum* is a plant of the family Aizoaceae.
- The plant is bitter, hot, alexiteric, analgesic, stomachic, laxative etc.
- METP revealed the presence of alkaloids, flavonoids, phenolics, steroids, tannins and saponins.
- METP were safe at limit dose 2500 mg/kg and 5000 mg/kg with no mortality.
- In both aspirin and pylorus ligated induced gastric ulcer in rat models, METP reduced the ulcer index thus showing the anti ulcerogenic activity.
- The antiulcer activity of METP on pylorus ligation induced gastric ulcer can due to antisecretory activity. The mechanism of protection against aspirin induced gastric ulcer can be attributed to 5-lipoxygenase pathway.

Author Profile

- **Dr. Prakash :** Currently working as Assistant Professor in the Department of Pharmacology, C.L. Baid Metha College of Pharmacy, Chennai, Tamilnadu, India. He has having 18 nos. of National and International journals; more than 20 nos. of presented as oral and poster presentations in National and International conferences; guided 10 M.pharm students and 20 B.pharm students.

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Pylorus induced ulceration is due to the stress induced on hydrochloric acid producing mechanism in the body system, resulting in ulceration of the gastric mucosa because of accumulation of gastric acid in the stomach.¹⁹ METP showed a marked reduction in the ulcer parameters studied on the test animals in a dose dependent manner in pH, gastric volume and total acidity.

In the present study, the anti-ulcerative effects of METP were investigated in aspirin-induced gastric ulcer model rats. Aspirin has been reported to reduce the gastric juice pH and increase the volume of gastric juice²⁰ or decrease the volume of gastric juice and its acid output.²¹ In the present study, the volume of gastric juice, total acidity and pH was increased by aspirin treated group and fully recovered by METP with aspirin treated group in dose dependent manner. Non steroidal anti inflammatory drugs (NSAID's) like aspirin induced gastric damage is possibly mediated through leukotrienes production and 5- lipoxygenase pathway.²²

CONCLUSION

The present study suggested that the antiulcer activity of METP on pylorus ligation induced gastric ulcer can due to antisecretory activity. The mechanism of protection against aspirin induced gastric ulcer can be attributed to 5-lipoxygenase pathway.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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ABBREVIATIONS

- METP:** Methanolic extract of *Trianthema portulacastrum*
TP: *Trianthema portulacastrum*
GUD: Gastric ulcer disease
NSAID: Non steroidal anti inflammatory drugs

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