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Research Article

**GASTROPROTECTIVE ACTIVITY OF METHANOLIC  
EXTRACT OF *PHYLLANTHUS ACIDUS* FRUIT AGAINST  
INDOMETHACIN-INDUCED GASTRIC ULCERS IN RATS**Vangala Kavitha<sup>1</sup>, Pathakala Naveen<sup>2,3</sup>, Ramavath Swathi<sup>1</sup>, Alikatte Kanaka Latha\*<sup>2</sup><sup>1</sup> Nethaji Institute of Pharmaceutical Sciences, Kakatiya University, Warangal, TS, India.<sup>2</sup> University College of Technology, Osmania University, Hyderabad – 500 007, TS, India.<sup>3</sup> Anurag Group of Institutions, School of Pharmacy, Venkatapur, Hyderabad – 500 088, TS, India.**Abstract**

*This study investigated the gastroprotective effect of methanolic extract of Phyllanthus acidus fruit (MPA) against indomethacin-induced gastric ulcer in rats. Ulceration was induced by a single oral administration of indomethacin (80 mg/kg body weight). Wistar rats were pre-treated with ranitidine (reference drug) at a dose of 40 mg/kg body weight and MPA at doses of 125 and 250 mg/kg body weight once daily for 21 days prior to ulcer induction. After 4 h of indomethacin administration, gastric secretions, antioxidant parameters and stomach nitric oxide (NO) were evaluated. The results showed that indomethacin induced gastric ulcer was associated with a significant increase of malondialdehyde and significant decrease of the gastroprotective mediators such as glutathione (GSH) and NO compared with normal control. Pre-treatment with MPA has shown improvements in indomethacin induced ulcers. In addition, MPA reduced oxidative stress parameters, free and total acidity and gastric NO content. Collectively, MPA produced gastroprotective effect in indomethacin induced gastric ulcers by anti-secretory action and cytoprotective effect.*

**Keywords:** Peptic ulcer, indomethacin, glutathione, Phyllanthus acidus, prostaglandins.**Corresponding author:****Alikatte Kanaka Latha,**University College of Technology,  
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## INTRODUCTION:

Ulcers are deep lesions penetrating through entire thickness of gastrointestinal mucosa and muscularis mucosae. Peptic ulcer is the most common among many types of ulcers [1]. Peptic ulcer is a lesion of gastric or duodenal mucosa. Peptic ulcer is a chronic, non-malignant inflammatory disease and has become almost a hall mark of the so called civilized life which causes a high rate of morbidity particularly in the population of non-industrialized countries [2]. Although potent anti-ulcer drugs are available; most of them produce several toxicities, thus emphasizing the need to search for new alternatives [3]. Natural medicines which are traditional play very important role in health care because 80% of drugs originated from natural sources [4].

The exact causes of peptic ulcer disease are not known but it may be produced when any factor causes an imbalance between the protective factors (mucus and bicarbonate) and aggressive factors (acid and pepsin) in the stomach. Such factors could range from natural causes like infections (*H. pylori*), non-steroidal anti-inflammatory drugs (NSAIDs), alcohol, stress and cigarette smoking [5]. Peptic ulcer disease occurs mainly due to consumption of NSAIDs, infection by *H. pylori*, stress or due to pathological condition such as Zollinger-Ellison Syndrome [6].

Long-term use of NSAIDs is the second most common cause of peptic ulcer disease after *Helicobacter pylori* (*H. pylori*) infection [7]. Blocking of prostaglandin synthesis through inhibition of the cyclooxygenase (COX) enzymes plays a cardinal role in the pathogenesis of NSAID-induced peptic ulcers [8]. However, there is indisputable evidence that other prostaglandin-independent mechanisms are also involved. These include generation of reactive oxygen species (ROS), initiation of lipid peroxidation and infiltration of neutrophils secondary to the production of inflammatory mediators such as tumour necrosis factor alpha (TNF- $\alpha$ ) and leukotriene's [9-11]. Indomethacin has been considered the drug of choice for the experimental induction of gastric ulcer [12].

Number of drugs, including proton pump inhibitors, prostaglandins analogs, histamine receptor antagonists and cytoprotective agents, are available for the treatment of peptic ulcer, require prolong period of intake [13]. Many drugs have various adverse effects [14] and no drug proves solely effective in treating peptic ulcer. Hence, herbal medicines are generally used in such cases when drugs are to be used for chronic periods. Several natural drugs have been reported to possess anti-ulcerogenic activity by virtue of their

predominant effect on mucosal defensive factors [15].

*Phyllanthus acidus* (*P. acidus*) is commonly known as star gooseberry. *P. acidus* (from the family Euphorbiaceae) plant is also one of the important plants having various medicinal properties such as antioxidants and anti-inflammatory effects [16]. *P. acidus* is consumed as herb by the Indian tribal for remedy of gastrointestinal tract disorders [17].

*P. acidus* has been used traditionally in the treatment of several pain, inflammatory and oxidative stress-related disorders such as rheumatism, bronchitis, asthma, respiratory disorder, hepatic disease, diabetes and gonorrhoea. The plant is also important to improve eyesight, memory and to cure cough, psoriasis, skin disorders, sudorific [18-20]. Fruits of the plant are used as astringent, root and seed are useful as cathartic and leaf and root are used as antidote to viper venom [21]. Methanolic extract of fruits and leaves was reported to show antimicrobial effect [22]. Petroleum ether extract of fruits was reported to show cytotoxic, antibacterial and antioxidant activities [23]. The fruits and leaves of the plant yielded promising hepatoprotective activity [24]. The methanolic fruit extract of the plant has been reported to show antibacterial, cytotoxic and antioxidant properties [25]. Hence, the present work was undertaken to investigate the gastroprotective activity against indomethacin induced ulcer.

## MATERIALS AND METHODS:

### Preparation of extracts

The collected fresh fruits of *P. acidus* weighing 5 kg were then washed properly to remove dirty materials and shade dried for several days with occasional sun drying. These were then dried in an oven for 24 h at considerably low temperature for better grinding. The grounded powder (500 g) was macerated with methanol and extracted by cold extraction process. The extract was concentrated to a small volume using rotary evaporator and allowed to dry. After drying, the extracts were weighed and percentage extractive values were determined.

### Animals

Young adult wistar rats (150-200 g) of both sexes were used. Animals were maintained at controlled room temperature (22 $\pm$ 3  $^{\circ}$ C) with free access to food and water, under a 12 h. light/dark cycle. Twenty-four hours before the experiments, they were transferred to the laboratory and given only water, ad libitum. The experiments were performed after approval of the protocol by the institutional animal ethics committee (IAEC) and were carried out in accordance with the current guidelines of

committee for the purpose of control and supervision of experiments on animals (CPCSEA.)

### Drugs and chemicals

Indomethacin was obtained from Ranbaxy Laboratories, India and ranitidine hydrochloride was obtained from GlaxoSmithKline, Mumbai, India. Other chemicals were of analytical grade.

### Experimental design

Albino rats of either sex were divided into five groups of six animals in each group:

Group I animals were not given with any drug and considered as control. Group II were given with indomethacin (IND) at a single oral dose of 80 mg/kg to induce gastric injury. Group III and IV received methanolic extract of *Phyllanthus acidus* (MPA) at the doses of 125 and 250 (mg/kg, p.o.), [26] whereas group V animals were treated with H<sub>2</sub> receptor antagonist such as ranitidine (RAN) (40 mg/kg, p.o.). Pre-treatment with the reference drug and extracts were given for 21 days prior to indomethacin administration. On day 21, all the animals were initially given with single oral dose of indomethacin and after 30 min. the remaining treatments (Extract and reference drugs) were given.

Rats were anesthetized 4 h. after indomethacin administration and the abdomens were opened with clamping of pylorus to collect gastric juice then their stomachs were removed, opened along the greater curvature, and washed with cold saline. Gastric juices were collected and centrifuged for 5 min. at 2000 rpm and collected supernatants. Also, the extent of gastric lesions (ulcer index, UI) was calculated by the formula [27]:

$$UI = 10 / (\text{total mucosal area} / \text{total ulcerated area})$$

Then the stomach of each rat was cut into multiple pieces for assessment of gastric mucosal damage.

### Determination of free, total acidity and pH of gastric juice

Gastric juice was collected, centrifuged and the supernatant was titrated with 0.01 N NaOH using methyl orange as an indicator until yellowish orange colour come out and the result indicated free acidity. Then phenolphthalein was added as an indicator and continues titrating until red colour reappears. The total volume of alkali added was considered as total acidity [28]. Moreover, pH of gastric juice was determined by digital pH meter.

#### Determination of free Acidity

$$\text{Acidity} = \frac{\text{Volume of NaOH} \times \text{Normality of NaOH}}{0.1} \times 100 \text{ mEq/l per } 100 \text{ gm}$$

### Evaluation of ulcer index (UI), percentage inhibition and ulcer score

Ulcer index (UI), percentage inhibition and ulcer score was calculated. For the determination of ulcer

index, stomachs were isolated, opened along the greater curvature and were gently rinsed with saline to remove gastric content and blood clot. Ulcer index was (UI) measured by following formula.

$$UI = U_N + U_S + U_P \times 10^{-1}$$

Where UI = Ulcer index

U<sub>N</sub>= Average number of ulcer per animal

U<sub>S</sub> = Average number of severity score

U<sub>P</sub>= Percentage of animals with ulcers

Percentage inhibition of ulceration was calculated as below [29]

$$\text{Percentage inhibition of ulceration} = \frac{\text{Ulcer index control} - \text{Ulcer index test}}{\text{Ulcer index control}} \times 100$$

The ulcer index was calculated according to the scoring method of Tan *et al.*, [30]

Score 0 = No ulcer

Score 1 = Vessel dilation and pointed ulcers

Score 2.5 = Small ulcers < 4 mm long

Score 5 = Large ulcers > 5 mm long

### Determination of glutathione (GSH) and malondialdehyde (MDA)

To evaluate the levels of glutathione (GSH) and malondialdehyde (MDA), 250 mg of stomach tissue was homogenized in 2.5 ml potassium phosphate buffer (pH 7.5) using a polytron homogenizer and then centrifuged at 4000 rpm for 15 min at 4°C. The concentration of reduced GSH in the stomach tissue homogenate was determined colorimetrically with method described by Beutler *et al.*, [31], reduction of 5,5'-dithiobis 2-nitrobenzoic acid (DTNB) by reduced GSH to give yellow product that was measured at 412 nm in a spectrophotometer. The concentration of MDA in stomach tissue homogenate was determined colorimetrically by Ohkawa *et al.*, [32]. In the protocol, thiobarbituric acid (TBA) reacts with MDA present in the sample [in acidic medium, at 95 °C for 30 min.] to form TBA-reactive products (TBARS). The absorbance of these pink products was then measured at 532 nm in the spectrophotometer. Levels were then calculated using a kit-provided formula and presented as nmol /g tissue.

### Determination of nitric oxide (NO)

To evaluate the nitric oxide (NO) levels, 250 mg tissue was homogenized in 2.5 ml ice-cold normal (0.9 %) saline. Thereafter, 1 ml absolute ethanol was added to 0.5 ml homogenate to precipitate the proteins and the samples were then centrifuged at 3000 rpm for 10 min. at 4 °C. The gastric nitric oxide was determined by measuring its nitrite (an indicator of original NO present). This method depends on reduction of nitrate to nitrite by

vanadium trichloride ( $VCl_3$ ) followed by addition of griess reagent [33]. In brief, a sample of homogenate supernatant (500  $\mu$ l) was mixed with an equal volume of  $VCl_3$  and of Griess reagent (0.2% naphthylethylenediamine and 2% sulphaniamide in 5% hydrochloric acid). After incubation at 37 °C for 30 min., the absorbance of the mixture was measured at 540 nm in the spectrophotometer [33]. Sodium nitrite standards assessed in parallel, values were compared to it, and the nitrite concentration in each sample was calculated and presented as nmol NO/g tissue.

#### Statistical analysis

The statistical data was analyzed by using one way analysis of variance (ANOVA) followed by Boenferoni multiple comparison test. Results were expressed in terms of Mean $\pm$ SEM for 6 animals in each group. *P* value <0.05 was considered statistically significant.

#### RESULTS:

##### Effect of MPA on ulcer index, ulcer score and percentage inhibition

The ulcerated indomethacin group showed ulcer score of 4.5 $\pm$ 0.23 and UI of 654.2 $\pm$ 7.9. Rat groups

treated with MPA (125 and 250 mg/kg, p.o.) or ranitidine (40 mg/kg, p.o.) prior to indomethacin showed a significant decrease of ulcer score, which was 3.2 $\pm$ 0.45, 2.1 $\pm$ 0.65 and 1.9 $\pm$ 0.32, respectively. Ranitidine (40 mg/kg, p.o.) showed a greater gastroprotective effect with UI of 260.3 $\pm$ 2.5 and preventive index 39.80%, while MPA (125 and 250 mg/kg, p.o.) showed UI of 559.2 $\pm$ 4.5, 430.4 $\pm$ 3.6 and a preventive index of 85.47, 65.74%. (Table. 1).

##### Effect of MPA on pH, free acidity and total acidity

Administration of indomethacin showed a significant decrease in gastric pH (1.83 $\pm$ 0.62) when compared to control group. Pre-treatment with MPA (125 and 250 mg/kg, p.o.) has shown a significant increase in gastric pH (2.40 $\pm$ 0.27, 3.13 $\pm$ 0.22) when compared to the control (3.71 $\pm$ 0.47) and reduced the total acidity (H<sup>+</sup> concentration) of acid secretion (70.83 $\pm$ 4.462, 65.83 $\pm$ 2.301). Ranitidine (40 mg/kg, p.o.) treated group has shown significant results, increase in gastric pH accompanied by a fall in total acidity when compared to the vehicle group. (Table 2).

**Table 1. Effect of MPA on ulcer index, ulcer score and percentage inhibition**

Group	Treatment	Ulcer Index	Ulcer Score	% Inhibition
Group I	Control	0	--	0
Group II	IND	654.2 $\pm$ 7.9*	4.5 $\pm$ 0.23*	NA
Group III	MPA (125 mg/kg, p.o.)	559.2 $\pm$ 4.5#	3.2 $\pm$ 0.45#	85.47#
Group IV	MPA (250 mg/kg, p.o.)	430.4 $\pm$ 3.6#	2.1 $\pm$ 0.65#	65.74#
Group V	IND+ RAN	260.3 $\pm$ 2.5#	1.9 $\pm$ 0.32#	39.80#

Values are presented as mean  $\pm$ SEM, n= 6. \**p*< 0.01 compared between control group and indomethacin group. # *p*<0.05 compared between treatment group with indomethacin group and control group. One way ANOVA followed by Boenferoni multiple comparison tests.

**Table 2. Effect of MPA on pH, free acidity and total acidity**

Group	Treatment	pH	Free acidity (meq/L) 100gm	Total acidity (meq/L) 100gm
Group I	Control	3.71 $\pm$ 0.47	43.33 $\pm$ 0.667	82.83 $\pm$ 1.108
Group II	IND	1.83 $\pm$ 0.62*	18.67 $\pm$ 0.337*	38.67 $\pm$ 0.988*
Group III	MPA (125 mg/kg, p.o.)	2.40 $\pm$ 0.27#	31.00 $\pm$ 3.425#	70.83 $\pm$ 4.462#
Group IV	MPA (250 mg/kg, p.o.)	3.13 $\pm$ 0.22#	29.83 $\pm$ 3.301#	65.83 $\pm$ 2.301#
Group V	IND+ RAN	3.67 $\pm$ 0.28#	30.67 $\pm$ 3.106#	66.67 $\pm$ 2.47#

Values are presented as mean  $\pm$ SEM, n= 6. \**p*< 0.01 compared between control group and indomethacin group. # *p*<0.05 compared between treatment group with indomethacin group and control group. One way ANOVA followed by Boenferoni multiple comparison tests.

##### Effect of MPA on oxidative stress markers

Indomethacin group showed a significant increase (195.98 $\pm$ 24.6) in gastric MDA but a significant decrease (11.24 $\pm$ 1.93) in gastric GSH when compared to control. Pre-treatment with either MPA (125 and 250 mg/kg, p.o.) or ranitidine (40 mg/kg, p.o.) showed a significant decrease in

gastric MDA when compared to ulcer control group 181.65 $\pm$ 14.6, 159.79 $\pm$ 13.5, 150.67 $\pm$ 12.1 respectively (Table. 3). A significant increase in gastric GSH was observed in both MPA (125 and 250 mg/kg, p.o.) and Ranitidine (40 mg/kg, p.o.) groups when compared to ulcer control group (21.27 $\pm$ 0.46, 31.83 $\pm$ 0.93, 33.24 $\pm$ 0.84 respectively).

**Effect of MPA on cytoprotective mediator (Stomach NO):**

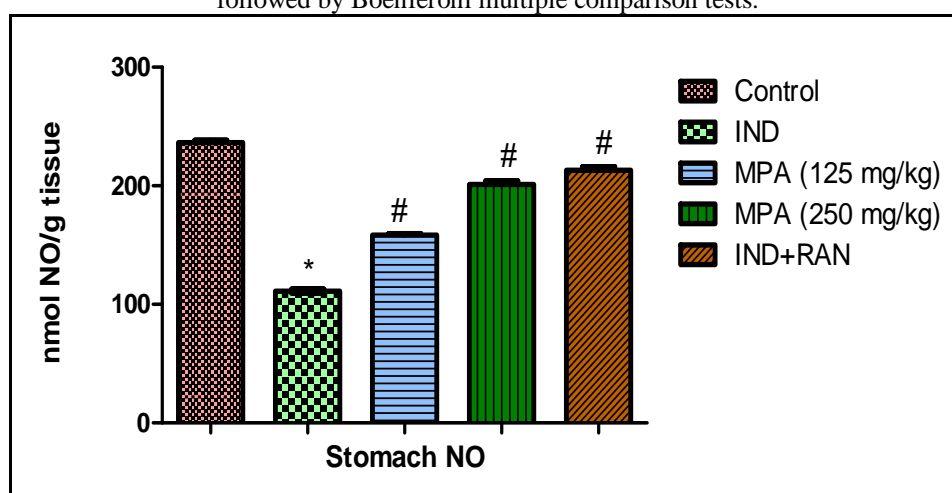
Gastric NO decreased significantly in indomethacin group when compared to normal control group (111.22±1.63) compared to indomethacin group,

the rats pre-treated with ranitidine (40 mg/kg, p.o.) or MPA (125 and 250 mg/kg, p.o.) exhibited a significant elevation of gastric NO (213.24±2.84, 158.27±1.36 and 201.13±2.93) (Figure.1.)

**Table 3. Effect of MPA on oxidative parameters**

Group	Treatment	LPO (nmol MDA/mg protein)	GSH (µmol/mg protein)
Group 1	Control	146.72± 11.2	36.08±0.15
Group 2	IND	195.98±24.6*	11.24±1.93*
Group 3	MPA (125 mg/kg, p.o.)	181.65±14.6#	21.27±0.46#
Group 4	MPA (250 mg/kg, p.o.)	159.79±13.5#	31.83±0.93#
Group 5	IND+ RAN	150.67±12.1#	33.24±0.84#

Values are presented as mean ±SEM, n= 6. \*p<0.01 compared between control group and indomethacin group. # p<0.05 compared between treatment group with indomethacin group and control group. One way ANOVA followed by Boenferoni multiple comparison tests.

**Figure 1. Effect of MPA on cytoprotective mediator (Stomach NO)**

Values are presented as mean ±SEM, n= 6. \*p< 0.01 compared between control group and indomethacin group. # p<0.05 compared between treatment group with indomethacin group and control group. One way ANOVA followed by Boenferoni multiple comparison tests.

**DISCUSSION:**

Ulcer has become a global disease affecting people in all geographical regions. It is generally accepted that peptic ulcer results from an imbalance between aggressive factors and protective factors; the maintenance of mucosal integrity through the endogenous defense mechanisms [34]

To regain the balance, different therapeutic agents are used to inhibit the gastric acid secretion or to boost the mucosal defence mechanisms by increasing mucosal production, stabilizing the surface epithelial cells or interfering with the prostaglandin synthesis [35]. Most commonly used NSAIDs such as indomethacin, aspirin etc. may cause gastrointestinal ulceration. Indomethacin (80 mg/kg, p.o) is commonly used for ulcer induction in experimental rats, which acts by suppression of prostaglandin synthesis and that leads to intense gastric mucosal damage [36, 37].

In the stomach, prostaglandins play a vital protective role, stimulating the secretion of bicarbonate and mucus, maintaining mucosal blood flow, and regulating mucosal cell turnover and repair [38].

In this model, MPA treated groups have shown changes in all gastric secretion parameters. We found that MPA (125 and 250 mg/kg, p.o.) reduced the total acidity, the volume of the gastric juice and increased gastric pH when compared to indomethacin group, indicating anti secretory effect of the MPA.

Free radicals play a critical part in pathophysiology mechanism of NSAIDs-induced peptic ulcer [39]. The present study revealed induction of gastric level of TBARs by oral treatment with indomethacin however these free radicals depleted GSH gastric content. Indomethacin inhibited the mitochondrial oxidative phosphorylation leading to the release of Cytochrome c from mitochondrial inter membranous space into cytosol and to the



release of ROS such as superoxide anion and H<sub>2</sub>O<sub>2</sub>. These free radicals declined the intracellular ATP concentration, leakage of Ca<sup>+2</sup> out of mitochondria, cellular osmotic imbalance and lipid peroxidation, resulting in increased permeability and subsequent mucosal damages [40]. Pre-treatment with MPA (125 and 250 mg/kg, p.o.) in this study reduced gastric levels of TBARs and increased GSH level.

Nitric oxide (NO) is a mediator of gastrointestinal mucosal defense but inconsistently, it also contributes to mucosal damage. Nitric oxide synthase (NOS) is responsible for synthesis of nitric oxide, which is present in many isoforms. Cytoprotective endothelial nitric oxide synthase (eNOS) and cytotoxic inducible nitric oxide synthase (iNOS) are two important isoforms of NOS [41]. Nitric oxide from eNOS improves the mucosal blood flow, protects the integrity of epithelial tissue and inhibits activation, adhesion and migration of leucocytes in the inflammatory [42] resulting in increasing mucus synthesis and accelerating ulcer healing [43]. This study showed down regulation of treatment with indomethacin to gastric tissue eNOS gene resulting in a decrement in gastric level of NO leading to a decrease in mucosal synthesis and mucosal barrier content which confirmed by biochemical and histopathological analysis. Meanwhile pre-treatment with MPA (125 and 250 mg/kg, p.o.) increased eNOS gene expression as well as gastric level of NO leading to increasing mucus synthesis plus restoration of the depleted gastric mucus levels.

### CONCLUSION:

In conclusion, oral treatment with MPA produced significant gastroprotective effects in indomethacin induced gastric ulcer by anti-secretory action and cytoprotective effect. Further studies to identify the active moieties and elucidation of the mechanism of action are recommended.

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