



Evaluation of Anti-Ulcer Activity of Ethanolic Extract of *Phyllanthus Reticulatus*

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ABSTRACT

The present study confirms the antiulcer activity of *Phyllanthus reticulatus* as it produced significant antiulcer property by their anti-secretory, cytoprotective and proton pump inhibitory properties. Further studies are needed to isolate the chemical moiety responsible for the antiulcer activity of this extract. Peptic ulcer is one of the most common gastrointestinal diseases. The exact causes of peptic ulcer disease are not known but it may be result from an imbalance between acid-pepsin secretion and mucosal defense factors. 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. *MurrayaKoenigii* commonly known as ‘meethi Neem’ in Hindi, belong to family Rataceae, is an aromatic deciduous shrub or a small tree up to 6m in height found throughout India. It commonly occurs in foothills of Himalaya, Assam, Skim, Kerala, Tamil Naidu, Andra Pradesh, and Maharashtra. On phytochemical investigation researcher claimed that leave of *MurrayaKoenigii* found to contain alkaloids, volatile oil, glycozoline, xanthotoxin, and sesquiterpenes. The leaf has been found to show antioxidant activity, hypoglycaemic activity, antibacterial activity, anti- dysentery, and act as a hepatoprotective

Keywords: Antiulcer activity, Ethanolic extract, Aspirin induced ulcer model, NSAIDs.

1. INTRODUCTION

Peptic ulcer is basically an inflamed break in the mucus membrane lining the alimentary tract. It is an excoriated area of stomach caused principally by the digestive action of gastric juice, upper small intestinal secretions. Prolonged use of non-steroidal anti-inflammatory drugs (NSAIDs) and *Helicobacter pylori* infection are two major factors that can disrupt the mucosal resistance. The incidence of duodenal ulcers has dropped significantly during the last few decades, while the incidence of gastric ulcers has shown a small increase in recent years, which is mainly caused by the widespread use of NSAIDs. The two most important developments associated with the overall decreased rates of peptic ulcer disease are the discovery of affective and potent acid suppressants and the identification of *H. pylori* as the main causative agent. In essence, as the infectious cause of gastric ulceration is being successfully fought, a higher percentage of the U.S. population is succumbing to gastritis and ulceration from the chronic consumption of medication, primarily NSAIDs.

Stress is an adaptive physiological response to disruption of body’s internal homeostasis. Excessive stress can induce organ injury or contribute to diseases, such as gastric ulcers, hypertension, diabetes, and cancer. Stress-induced gastric ulceration is a typical example of stress-associated organ injuries. Acute gastric mucosal injury is a serious clinical problem worldwide. Since the development of gastric lesions during restraint stress was significantly enhanced by exposure to water immersion. The rise in acid secretion may be an important aggravating factor in the process of ulceration during water immersion. The use of herbal medicine has become increasingly popular worldwide and medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects. Recently, it has also been reported that many traditional Asian herbal remedies act on gastro-intestinal diseases.

The use of nonsteroidal anti-inflammatory drugs (NSAIDs), aspirin, has been expanded to include the prophylaxis of cardiovascular and cerebrovascular diseases. However, treatment with low dose aspirin may be toxic to the gastrointestinal tract. NSAIDs including aspirin are known to induce gastric mucosal damage and delay ulcer healing, most likely by inhibiting cyclooxygenase (COX) activity and reducing gastric PG levels. Angiogenesis, a phenomenon involving the formation of new vessels from the pre-existing microvasculature, is an essential component of the recovery from ischemic conditions, and it is mainly regulated by angiogenic growth factor such as vascular endothelial growth factor (VEGF)-A. The healing of gastric ulcer is highly dependent on angiogenesis. VEGF has been suggested to play an important role in gastric ulcer healing.

A large number of spices and herbs have been evaluated by various researchers for their antiulcer effects to achieve a favorable outcome. Large numbers of medicinal plants and dietary nutrients have been shown to possess gastro-protective activities such as Aloe, Terminalia Chebula, VetiveriaZiziinoides, Ginseng, Capsicum etc. Despite being one of the well-known medicinal plants used in Indian traditional medicine to treat several ailments, studies pertaining to the pharmacological properties of some medicinal plants are very scarce.

Since ancient times, plants and plants derived products have been used in folklores around the world for the treatment of several ailments and diseases. Nowadays, herbal medicine is becoming a viable alternative treatment over the commercially available synthetic drugs on Peptic ulcer management/treatment. This is premised on its lower cost, perceived effectiveness, availability as well as little or no adverse effects. A number of these herbal remedies have demonstrated gastroprotective properties and have been used in the treatment of Peptic ulcer, digestive disorders, and other related ailments for several centuries.

Ulcer is one of the most common diseases affecting throughout the world population. The allopathic treatment of ulcer adversely affects the health by causing harmful side effects. Currently, many herbal plants and secondary metabolites have been used for the ulcer treatment. In the present review, many herbal plants, and their parts (root, rhizome, bark, leaves and fruits) have been listed in the table are currently being used for ulcer treatment. Recently, medicinal plants have emerged as efficacious, safe, and widely available alternative therapies for peptic ulcer disease. The aim of this review was to study the medicinal plants and phytochemicals, which have been used for peptic ulcer disease treatment to evaluate the potential role of natural compounds to develop herbal remedies for peptic ulcer disease.

Phyllanthus reticulatus, family Euphorbiaceae is a climbing shrub which grows all over India. It is a monoecious scandent shrub or small bushy tree having height 8 to 10 ft. Fruits are depressed-globose berry, up to 7 mm in diameter, usually bluish-black when ripe with dark purplish pulp. The plants of this genus were reported to contain lignans, flavonoids, triterpenoids, alkaloids, polyphenolic compounds. Flavonoids and polyphenolic compounds have a potential role in the prevention of various diseases through their antioxidant activity. The plant is used for a variety of ailments, including smallpox, syphilis, asthma, diarrhea, and bleeding from gums. Moreover, it is also claimed the plant has anti-diabetic activity in tribal area. This plant also showed hypotensive, hypocholesterolemia effects and its folkloric use in gastric complaints including colic, constipation etc.

CLASSIFICATION:

A. By region/area:

1. Duodenum (called duodenal ulcer)
2. Esophagus (called esophageal ulcer)
3. Stomach (called gastric ulcer)
4. Meckel's diverticulum

B. Modified Johnson classification of peptic ulcers:

- **Type I:** Ulcer along the body of the stomach, most often along the lesser curve at incisura angularis along the locus minor is resistant. Not associated with acid hypersecretion.
- **Type II:** Ulcer in the body in combination with duodenal ulcers. Associated with acid over secretion.
- **Type III:** In the pyloric channel within 3 cm of pylorus. Associated with acid over secretion.
- **Type IV:** Proximal gastroesophageal ulcer.
- **Type V:** Can occur throughout the stomach associated with chronic use of NSAIDs (such as aspirin).

2. MATERIAL AND METHOD

2.1 Plant Selection-

Drug discovery from medicinal plant includes numerous fields of inquiry & use various method of analysis. According to the intensive literature survey, Phyllanthus reticulatus was used for the present study.

In the traditional system of medicine, the plant is used as a remedy of gastrointestinal disorders. The ethanol-induced gastric ulcer model is widely used to evaluate the gastric cytoprotective effect of plant extract against ingested irritants. The medicinal plants signify a massive basin of potential phytoconstituents that could be valuable as a substitute to allopathic drugs or considered as an analogue in drug development. Phyllanthus reticulatus. (Euphorbiaceae) is generally used in traditional medicine to treat ulcer and inflammation. The present study confirms the antiulcer activity of Phyllanthus reticulatus as it produced significant antiulcer property by their anti-secretory, cytoprotective and proton pump inhibitory properties. Further studies are needed to isolate the chemical moiety responsible for the antiulcer activity of this extract.

Phyllanthus emblica L. (Phyllanthaceae) is an important medicinal and edible plant distributed across tropical and subtropical regions and has been listed as one of the three health-related plants to be promoted for planting around the world by the World Health Organization. From an ecological perspective, the tree is extremely resistant to drought and barren environments and can be used as a pioneer tree to establish forests on barren hills. The focus of recent research has been on the biochemistry and pharmacology of Phyllanthus. emblica.

Phyllanthus (Euphorbiaceae) species are traditionally well-known for their medicinal properties including hepatoprotective activity. The plant has a long history of use in traditional medicine. Various parts of the plant have been shown to contain tannins, which are partly responsible for its medicinal and dyeing properties:

- A number of triterpenoids have been isolated from the stems and leaves, including sitosterol, friedelin and betulinic acid.

- The stem bark contains pentacosane, 21- α -hydroxyfriedelan-3-one, taraxerol and lupene-24-diol.
- Petroleum ether and ethanol extracts of the leaves have shown hypoglycaemic effects.
- An ethanolic extract of the stem bark showed in-vitro antiviral properties against polio and measles viruses, and antitumor activity.
- Extracts of the leaves have shown promising antiplasmodial activity against chloroquine-resistant and -sensitive malaria parasites.
- Sap from the stem is blown into the eyes to cure soreness.
- A soup made of the leaves, boiled with palm fruits, is given to women after child-birth.
- The powdered leaves are combined with cubebs and camphor then made into tablets that can be sucked in order to treat bleeding gums.
- The powdered leaf is used externally for topical application to sores, including venereal sores, burns, suppurations and skin-chafes.
- The mashed leaves are rubbed over the body of a malaria patient.
- The leaves and bark are reputed to be diuretic and cooling.
- The root is purgative and has a variety of uses.
- A decoction is used in the treatment of hookworm, whilst water in which the root has been boiled is taken as a male aphrodisiac, to increase fertility, to treat headache, for dysmenorrhoea, for hard abscesses.
- A decoction of the root, combined with the leaf-sap, is used as an antispastic.
- The plant is considered a remedy for anaemia and intestinal haemorrhage.

2.2 Collection and identification-

The bark of *Phyllanthus reticulatus* was purchased from Botanical Garden of M.G. Science, Ahmedabad (GJ) and the plant samples were authenticated by Dr. S.N. Dwivedi, A.P.S. University Rewa.

2.3 Preparation of extracts

Fresh barks were collected and dried in shade at room temperature. The dried bark was coarse powdered, and the powder was packed in to Soxhlet column and extracted successfully with 70% ethanol at 60°C. The extracts were concentrated by using rotary flash evaporator under reduced pressure. The dried extracts were stored in air container in refrigerator below 100C.

- The solutions of ethanolic extract were prepared using distilled water.
- The ethanolic extract of bark of *Phyllanthus reticulatus* was subjected to the following studies
 1. Preliminary phytochemical screening.
 2. Antiulcer activity: Aspirin induced ulcer model.

2.4 Preliminary phytochemical screening

The preliminary phytochemical investigation was carried out for the ethanolic extract of *Phyllanthus reticulatus* for the detection of various phytoconstituents. Tests for the presence of common phytochemicals were carried out by following standard methods as described in practical pharmacognosy by K.R. Khandelwal.

2.5 Animals used

Wistar Albino rats weighing 150-200 g of either sex were used in the present study. The animals were allowed for acclimatization for ten days under laboratory conditions. They were housed in polypropylene cages and maintained at 27°C±20C, relative humidity 65±10% under 12 hrs light/dark cycles. The animals were fed with rodent pellet diet and water ad libitum. The study protocol was approved from the Institutional Animal Ethics Committee (IAEC) of SVCP.

2.6 Anti-ulcer activity: Aspirin induced ulcer model

Albino rats of either sex weighing between 150-200 g were selected and divided into 5 groups.

Group I – Vehicle Control

Group II – Negative Control (Aspirin 200 mg/kg)

Group III – Standard (omeprazole 8 mg/kg p.o.)+ Aspirin

Group IV – Ethanolic extract of *Phyllanthus reticulatus* 200 mg/kg p.o. + Aspirin

Group V – Ethanolic extract of *Phyllanthus reticulatus* 400 mg/kg p.o. + Aspirin

Food was withdrawn for 24 hrs before the experiments and water was allowed aspirin 200mg/kg b.w. was administered to produce ulcers. The test drugs were administered orally 30 minutes prior to omeprazole challenge. After 4 hrs animals were sacrificed, stomach dissected out for scoring ulcer index by histopathology.

The ulcer scoring done as below:

Ulcer Score	Description
0	Normal colored
0.5	Red coloration
1	Spot ulcers
1.5	Haemorrhagic streaks
2	Ulcers > 3 mm but < 5 mm
3	Ulcer > 5 mm

Mean ulcer score for each animal is expressed as ulcer index.

2.7 The percentage protection was calculated using the formula

$$\text{Percentage protection} = 1 \frac{U_t}{U_c} \times 100$$

Where,

U_t = ulcer index of treated group

U_c = ulcer index of control group.

The result was expressed as mean SEM and subjected to statistical analysis Using ANOVA followed by *Turkey Kramer* test.

2.8 Determination of free acidity and total acidity

1 ml of gastric juice was pipette out into 100 ml conical flask, added 2-3 drops of topfer's reagent and titrated 0.01 N sodium hydroxide until all traces of red colour disappears and the colour of the solution turns to yellowish orange. The volume of the alkali added was noted. This volume corresponds to free acidity. Then 2-3 drops of phenolphthalein solution were added, and titration was continued until a definite red tinge reappears. Again, the total volume of alkali added was noted. The volume corresponds to total acidity. The results are tabulated in

Acidity was calculated by using the formula

$$\text{Acidity} = \frac{\text{Volume of NaOH} \times \text{Normality of NaOH} \times 100}{0.1} \text{ meq/L/100gm}$$

3. RESULTS AND DISCUSSION

Effect of ethanolic extract of Phyllanthus reticulatus. (Twice daily for 5 days) on aspirin-induced gastric ulcers:

The ethanolic extract of *Phyllanthusreticulatus* was administered to various groups, orally, twice daily for 5 days and experiment were carried out on 18-24 hrs fasted rats on 6th day. Ulcer were scored and analysed as described earlier. The result indicated a dose dependent anti ulcerogenic activity from 21.1+ 1.6 mm²/rat aspirin induced gastric ulcer to 2.0 + 0.6 mm²/rat ($p < 0.001$) *Phyllanthusreticulatus* (400mg). The present study of ethanolic extract of *Phyllanthus reticulatus* was effective in synthetic NSAIDs like aspirin causes mucosal damage by interfering with prostaglandin synthesis, enhance acid secretion, increase back diffusion of H⁺ ions, and breaking of the mucosal barrier.



A. Gastric lesion induced by aspirin at 200mg/kg dose



B. Absence of gastric lesion in omeprazole 8mg/kg dose

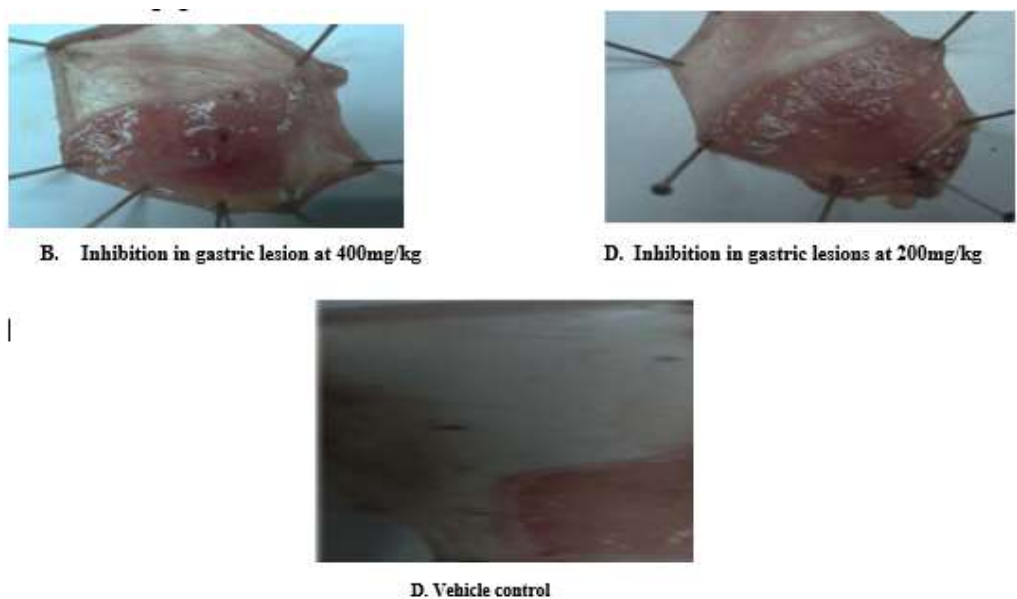


Fig. 1: Open excised stomach in aspirin induced gastric ulcer in rat

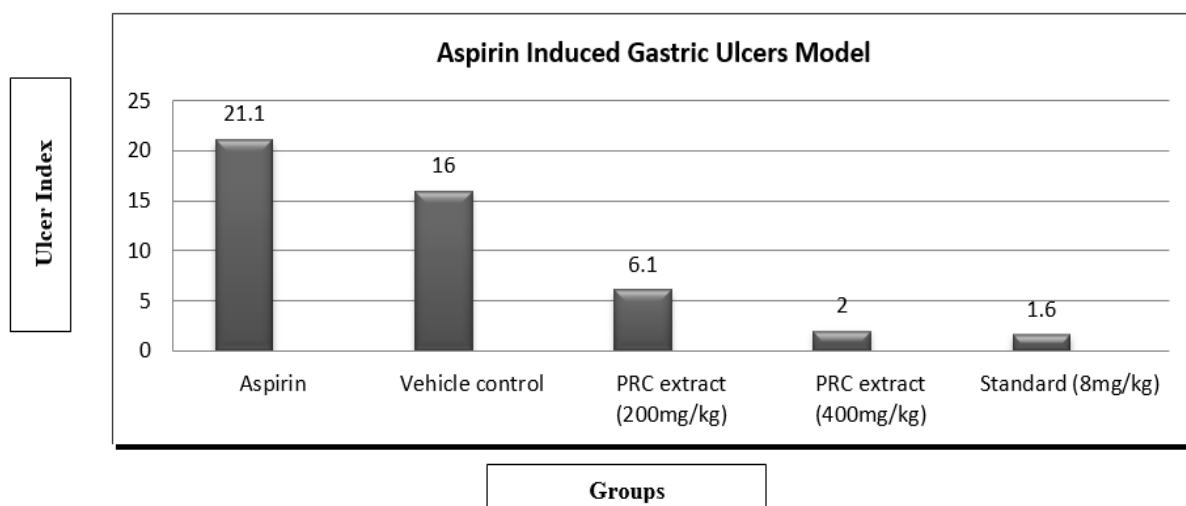
Table 1: Effect of ethanolic extract of *Phyllanthus reticulatus* (Twice daily for five days) on Aspirin - Induced Gastric ulcers.

Group	Treatment	Dose (mg/kg)	Ulcer Index (mm ² /rat)	% Protection
I	Aspirin	200	21.1 + 1.6	-
II	Vehicle control	200	16 + 0.92	-
III	PRC extract + Aspirin	200	6.1 ± 1.0 ^b	69.21
IV	Standard (Omeprazole) + Aspirin	8	1.6 ± 0.2	92.26
V	PRC extract + Aspirin	400	2 ± 0.37	70.73

Value is mean ± Σ for 6 rats.

a= p<0.05 compared to respective pylorus Aspirin group.

b= p>0.001 compared to respective pylorus Aspirin group.



Trend. 1: Effect of ethanolic extract of *Phyllanthus reticulatus* against aspirin-induced gastric ulcer

4. CONCLUSION

The present study confirms the antiulcer activity of *Phyllanthus reticulatus*. Further studies are needed to isolate the chemical moiety responsible for the antiulcer activity of this extract.

According to the old hypothesis, acid secretion was thought to be the sole cause of ulcer formation and reduction in acid secretion was thought to be the major approach towards therapy. However, in the light of recent evidence this concept has changed. Now, treatment of ulcer mainly targets the potentiation of the defensive system along with lowering of acid secretion. Chemical substances derived from plants have been used to treat human diseases since the dawn of medicine. Roughly 50% of new chemical entities introduced during the past two decades are from natural products. Recent technological advances have renewed interest in natural products in the drug discovery. Therefore, efforts should be directed towards isolation and characterization of the active principals and elucidation of the relationship between structure and activity furthermore, detailed analysis of the active constituents of natural drugs should be directed towards clinical relevance. Although the clinical efficacy of this extract is reported by traditional practices, they have not been scientifically validated. Ayurveda, the oldest medicinal system in the world provides leads to find therapeutically useful compounds from plants. Therefore, ayurvedic knowledge supported by modern science is necessary to isolate, characterized and standardize the active constituents from herbal source. This combination of traditional and modern knowledge can produce better anti-ulcer drug with fewer side effects. Herbs are widely available in India and other countries. The wide spectrum makes them attractive candidates for further research. Plant showed significant antiulcer activity which is evident by the data obtained.

Phyllanthus reticulatus a tremendous potential deserves a special attention of the scientific fraternity emerge as a milestone for medical science of this millennium due to its safety profile and can be a potent natural and safe alternative to conventional antiulcer treatment. However, there is a shortage of clinical trial regarding its potency and efficacy.

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