

Full Length Research Paper

Gastroprotective effect of *Phyllanthus niruri* leaf extract against ethanol-induced gastric mucosal injury in rats

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The main objective of this study is to evaluate the gastroprotective activity of the *Phyllanthus niruri* leaf extract against ethanol-induced gastric mucosal injury in rats. Six groups of Wistar rats were pre-treated, respectively, with distilled water; omeprazole 20 mg/kg; and 250, 500, 750 and 1000 mg/kg *P. niruri* leaf extract 30 min before oral administration of absolute ethanol to generate gastric mucosal injury. After one hour later, the rats were sacrificed and the ulcer areas of the gastric walls were determined. Gross evaluation has revealed that the negative control rats exhibited severe mucosal injury, whereas, pre-treatment with *P. niruri* leaf extract resulted in significantly less gastric mucosal injury and flattening of the mucosal folds. Histological studies of the gastric wall revealed that negative control rats suffered very severe damage of gastric mucosa, along with edema and leucocytes infiltration of the submucosal layer compared to rats pre-treated with *P. niruri* leaf extract where there was marked gastric protection along with reduction or inhibition of edema and leucocytes infiltration of the submucosa. The present finding suggests that *P. niruri* leaf extract promotes ulcer protection as ascertained by the comparative decreases in ulcer areas, inhibition or reduction of edema and leukocyte infiltration of the submucosa.

Key words: *Phyllanthus niruri*, rat, gastroprotection, ulcer.

INTRODUCTION

Phyllanthus niruri (Euphorbiaceae) is a small herb distributed throughout the tropical and subtropical regions of both hemispheres. This plant is popular in folk medicine, whole plant, fresh leaves and fruits are used in the treatment of various diseases, particularly hepatitis and other viral infection (Chopra et al., 1986; Wang, 2000).

The plant is of medicinal importance for numerous ailments like dysentery, influenza, vaginitis, tumors, diabetes, diuretics, jaundice, kidney stone, dyspepsia, antihepatotoxic, antihepatitis-B, antihyperglycemic and also as antiviral and antibacterial (Chopra et al., 1986). *P. niruri* extract has been shown to inhibit DNA polymerase

of hepatitis B virus and related hepatitis viruses (Blumberg et al., 1990) and the extract of this plant contains several bioactive molecules such as lignans, phyllanthin, hypophyllanthin, flavonoids, glycosides and tannins (Rajeshkumar et al., 2002). *P. niruri* was used for treating liver ailment (Kapur et al., 1994) and possess hypolipidemic (Khanna et al., 2002), antiviral (Jayaram et al., 1997), anticarcinogenic (Rajeshkumar et al., 2002), antioxidant (Harish and Shivanandappa, 2006), antinociceptive and antispasmodic activities as well as its role in the inhibition of calcium oxalate formation in kidney (Santos et al., 1995, Qian-Cutrone et al., 1996, Freitas et al., 2002).

In addition, recent studies have isolated some of the bioactive molecules from *P. niruri* and other types of plants (Kotoky et al., 2005; Cipriani et al., 2008) which may have an important role in homemade and industrial medicines for effective treatment of some pathological

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conditions. Another study (Shokunbi and Odetola, 2008) has reported that *Phyllanthus amarus* revealed gastroprotective and antioxidant activities on absolute ethanol induced ulcer in albino rats. There were no reports regarding the gastroprotective activity of *P. niruri*. The present study was undertaken in rats to evaluate for any anti-ulcerogenic properties from the aqueous extracts of *P. niruri*.

MATERIALS AND METHODS

Omeprazole

Omeprazole is a class of drugs called proton pump inhibitors used for the treatment of conditions such as peptic ulcers. Omeprazole blocks the enzymes in the wall of the stomach from producing acid. By blocking these enzymes, the production of stomach acid is decreased, thus, allowing the stomach to heal. In this study, omeprazole was used as the reference anti-ulcer drug, and was obtained from the University of Malaya Medical Centre (UMMC) Pharmacy. The drug was administered orally to the rats in concentrations of 20 mg/kg of body weight suspended in distilled water (5 ml/kg) (Pedernera et al., 2006).

Plant specimen and extract preparation

Fresh *P. niruri* leaves were purchased from Ethno resources (Sungai Buloh, Selangor, Malaysia), and identified by comparison with the voucher specimen deposited at the Herbarium of Rimba Ilmu, Institute of Science Biology, University of Malaya, Kuala Lumpur. The leaves were washed with distilled water, and then dried in shade for seven to ten days being ground to powder. Four hundred grams of the leaf powder was added to 8000 ml of sterile distilled water (1:20) in a conical flask. This mixture was heated and stirred on a hot plate for three hours. The residue was removed by filtration using a filter funnel and distilled under reduced pressure in an Eyela rotary evaporator (Sigma-Aldrich, USA). The extract was then freeze-dried and dissolved in distilled water for administration to rats in concentrations of 250, 500, 750 and 1000 mg/kg body weight (5 ml/kg).

Experimental animals

Adult male albinos Wistar rats were obtained from the Animal House, Faculty of Medicine, University of Malaya, Kuala Lumpur (Ethics No. PM 28/9/2007 MAA (R)). The rats weighed between 180 - 220 g. They were fasted for 48 h before the experiment (Garg et al., 1993), but were allowed free access drinking water for around two hours before the experiment. During the fasting period, the rats were placed individually in separate cages with wide-mesh wire bottoms to prevent coprophagy.

Acute toxicity test

The acute toxic class method of the Organization for Economic Cooperation and Development (OECD) was used to determine a safe dose for the extracts. Thirty animals (15 males and 15 females) were assigned equally into three groups labeled as vehicle (distilled water); 2 and 5 g/kg of leaf extract preparation, respectively. The animals were fasted overnight (food but not water) prior dosing. Food was withheld for a further three to four hours after dosing. Observations were done on mortality and behavioral

changes of the rats following treatment. All surviving animals were sacrificed after 24 h for gross and histopathological examination. This study was conducted according to the OECD Guideline 425 (OECD, 2001.).

Treatment and tissue sample collection

The rats were randomly divided into 6 groups with 6 rats in each group. Group 1 rats were negative controls that received 5 ml/kg distilled water orally by orogastric intubations; whereas, Group 2 rats received oral doses of 20 mg/kg omeprazole in (5 ml/kg) as positive controls. Group 3, 4, 5 and 6 rats received oral doses of 250, 500, 750 and 1000 mg/kg of aqueous leaf extract (5 ml/kg) by the same route, respectively. Thirty minutes after pre-treatment, gastric ulcers were induced with absolute ethanol (5 ml/kg) in all rats. Gastric ulcer was induced in the rats according to the method of Robert et al. with slight modification (Robert et al., 1979). The rats were euthanized 60 min later (Paiva et al., 1998) by overdoses of diethyl ether and their stomachs were immediately excised. Each stomach was opened along the greater curvature, washed with still water and fixed in 10% buffered formalin for 15 min.

Gross gastric lesions evaluation

Any ulcers would be found in the gastric mucosa, appearing as elongated bands of hemorrhagic lesions parallel to the long axis of the stomach. Each gastric mucosa was thus, examined for damage. Ulcer areas of gastric mucosa were calculated according to the method of Kauffman and Grossman (Kauffman and Grossman., 1978) with slight modification. The length (mm) and width (mm) of the ulcer on the gastric mucosa were measured by a planimeter ($10 \times 10 \text{ mm}^2 = \text{ulcer area}$) under dissecting microscope ($\times 1.8$). The area of each ulcer lesion was measured by counting the number of small squares, $2 \times 2 \text{ mm}$, covering the length and width of each ulcer band. The sum of the areas of all lesions for each stomach was applied in the calculation of the ulcer area (UA) where the sum of small squares $4 \times 1.8 = \text{UA mm}^2$. The inhibition percentage (%) was calculated by the formula of (Njar et al., 1995):

$$(\%) = [(UA_{\text{control}} - UA_{\text{treated}}) / UA_{\text{control}}] \times 100\%.$$

Histological evaluation of gastric lesions

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 μm and stained with hematoxylin and eosin for histological evaluation.

Statistical analysis

All values were reported as mean \pm S.E.M. The statistical significance of differences between groups was assessed using one-way ANOVA. The Mann-Whitney U test was used to compare the difference between two groups. A value of $p < 0.05$ was considered significant difference between the groups. Statistical computations were calculated using SPSS 11.5 for Windows software (SPSS Inc, Chicago, IL, USA).

RESULTS

Acute toxicity study

All treated animals were closely observed for any

behavioral changes, abnormal or toxic manifestations and for mortality up to 24 h. There were no mortality that occurred amongst the rats administered *P. niruri* leaf extracts with dose levels of 2 and 5 g/kg body weight during the period of study. Acute toxicity studies showed that plant extract was found to be safe up to maximum dose of 5 g/Kg body weight of the animal.

Gross evaluation of gastric lesions

Negative control rats exhibited severe mucosal injury whereas, pre-treated with *P. niruri* leaf extracts before being given absolute alcohol had significantly reduced areas of gastric ulcer formation with showing flattening of gastric mucosal folds compared to rats pre-treated with only distilled water (Table 1, Figures 1 and 2). There were no significant differences between 750 and 1000 mg/kg leaf extract in terms of ulcer area. It was also observed that protection of gastric mucosa was more prominent in rats pre-treated with 1000 mg/kg leaf extract (Table 1).

Histological evaluation of gastric lesions

The control rats pre-treated with only distilled water suffered markedly extensive damage to the gastric mucosa, edema and leucocytes infiltration of the submucosal layer. Rats that received pre-treatment *P. niruri* extract had comparatively better protection of the gastric mucosa as seen by reduction in ulcer area, reduced submucosal edema and absence of leucocytes infiltration. Such protection was markedly more prominent at 1000 mg/kg doses (Figures 3 and 4).

DISCUSSION

The method of absolute ethanol-induced gastric mucosal damage is a rapid and convenient way of screening plant extracts for anti-ulcer potency and the level of gastro-protection could be determined in terms of absence or reduction in grossly visible gastric mucosal lesions. Absolute ethanol is highly corrosive to the gastric mucosa and its mechanism of action on rat gastric mucosa involves superficial necrosis of gastric mucosa and release of histamine and leucotriene C4 as tissue-derived mediators.

These mediators act on gastric microvasculature and result in destruction of mucosa and submucosa of gastric wall (Oates and Hakkinen, 1985). Our results demonstrate that oral administration of *P. niruri* extracts could significantly protect the gastric mucosa from ulcer induction by absolute ethanol, and the gastric mucosal protection improved as the doses were increased. The gastro-protective effects of *P. niruri* may be attributed to various compounds present in the plant, including acidic

heteroxylyan and some others polysaccharides present in the herb (Cipriani et al., 2008). Oxidative stress plays an important role in the pathogenesis of various diseases including gastric ulcer, with antioxidants being reported to play a significant role in the protection of gastric mucosa against various necrotic agents (Trivedi and Rawal, 2001). Studies on *Phyllanthus amarus* have showed the *in vivo* gastroprotective and antioxidant activities of acetone and aqueous gastric ulcer (Oluwole et al., 2002; Shokunbi and Odetola, 2008).

Antioxidants could help to protect cells from damage caused by oxidative stress while enhancing the body's defense systems against degenerative diseases. Administration of antioxidants inhibits ethanol-induced gastric injury in rat (Ligumsky et al., 1995) and *P. niruri* extract possess a broad spectrum of biological activities. *P. niruri* has been reported to possess antioxidant activities (Harish and Shivanandappa, 2006) and it is speculated that the gastro-protective which was exerted by *P. niruri* leaf extract could be attributed to its antioxidant property. *P. niruri* extracts showed inhibition of membrane lipid peroxidation, potent free radical scavenging and inhibition of reactive oxygen species, and this could associate with its high medicinal value (Harish and Shivanandappa, 2006). Phytochemical analysis of the *P. niruri* extract showed the presence of several bioactive molecules such as lignans, phyllanthin, hypophyllanthin, flavonoids, glycosides and tannin have been shown to be present in the *P. niruri* extracts (Rajeshkumar et al., 2002). Any one of the observed phytochemical constituents present in *P. niruri* extract may be responsible for their gastro-protective activity. Phytochemical constituents like flavonoids (Tsuchiya et al., 1996) and triterpenoids (Scortichini and Rossi, 1991) are known to prevent gastric ulcer due to the astringent and antimicrobial properties, which appear to be responsible for gastro-protective activity. The gastro-protective activity of *P. niruri* extracts may be attributed to the phyto-constituents present in the plant. The prevention of gastric ulcer in our study may have been attributed by the tannin phyto-constituent of *P. niruri* from the stringent effect which has been reported elsewhere (Chaudhari and Mengi, 2006).

The result of the present study also revealed protection of gastric mucosa and inhibition of leucocytes infiltration of gastric wall. Kobayashi et al. (2001) observed protection of gastric mucosa and inhibition of neutrophil infiltration, and Shimizu et al., (2000) reported that reduction of neutrophil infiltration into ulcerated gastric tissue promote the healing of gastric ulcers in rats, and such increase in neutrophil infiltration into ulcerated gastric tissue could delay the healing of gastric ulcers in rats (Fujita et al., 1998). Flattening of the mucosal folds which suggests that gastroprotective effect of *P. niruri* extract might be due to a decrease in gastric motility. It is reported that the changes in gastric motility may play a role in the development and prevention of experimental gastric lesions (Mersereau and Hinchey., 1982; Takeuchi and

Table 1. Observed ulcer area and inhibition percentage in rats.

Animal group	Treatment (5 ml/kg dose)	Ulcer area (mm ²) (Mean ± S.E.M)	Inhibition (%)
1	Distilled water (Control)	940.00 ± 20.00	-
2	Omeprazole (20 mg/kg)	130.00 ± 5.77a	86
3	<i>P. niruri</i> (250 mg/kg)	210.00 ± 10.65**	77.66
4	<i>P. niruri</i> (500 mg/kg)	140.00 ± 5.77a	79.11
5	<i>P. niruri</i> (750 mg/kg)	50.00 ± 2.89*	85.12
6	<i>P. niruri</i> (1000 mg/kg)	No ulcer was seen	100.00

All values are expressed as mean ± S.E.M. * $P < 0.001$ vs. Group 2, Group 3, and Group 4. ** $P < 0.01$ vs. Group 4. a No significant difference between these two groups.



Figure 1. Gross appearance of the gastric mucosa in a rat pre-treated with only distilled water (negative control).
Note: Severe injuries are seen in the gastric mucosa.

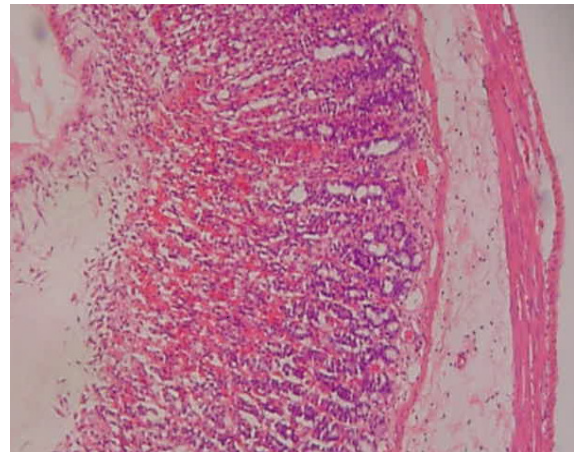


Figure 3. Histological section of gastric mucosa in a rat pre-treated with only distilled water.
Note: There is severe disruption to the epithelium surface and deep mucosa. Leukocyte infiltration and edema are observed in the submucosa layer (H&E stain, 40 x magnifications).



Figure 2. Gross appearance of the gastric mucosa in a rat pre-treated with 1000 mg/kg of *P. niruri* extract.
Note: No ulcerations are seen in the gastric mucosa and the gastric mucosa is flattened.

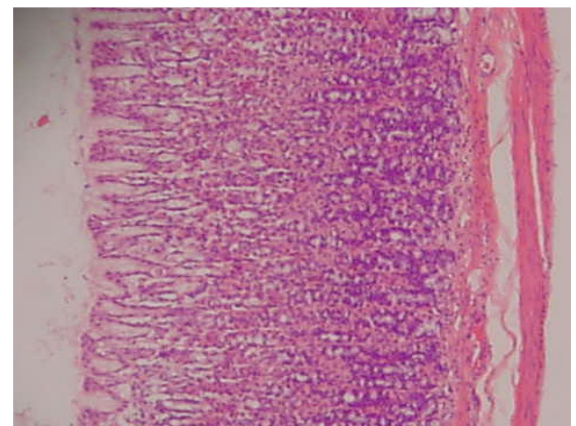


Figure 4. Histological section of gastric mucosa in a rat pre-treated with 1000 mg/kg of *P. niruri*.
Note: There is no disruption to the surface of epithelium with neither edema nor leukocytes infiltration of the submucosal layer (H&E stain, 40x magnification).

Nobuhara, 1985). Relaxation of circular muscles may protect the gastric mucosa through flattening of the folds. This will increase the mucosal area exposed to necrotising agents and reduce the volume of the gastric irritants on rugal crest. Such action has been postulated to play a role in cytoprotective effect of prostaglandin (Takeuchi and Nobuhara, 1985). Ethanol produces a marked contraction of the circular muscles of rat fundic strip. Such a contraction can lead to mucosal compression at the site of the greatest mechanical stress, that is, at the crests of mucosal folds leading to necrosis and ulceration (Mersereau and Hinchey, 1982).

In conclusion, gastroprotective activity leaf extracts could significantly protect the gastric mucosa against ethanol-induced injury. Such protection was shown to be dose dependent as ascertained by the reduction of ulcer areas in the gastric wall as well as the reduction or inhibition of edema and leucocytes infiltration of submucosal layers, and such protection are most prominent at a dose of 1000 mg/kg leaf extract.

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