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Gastro-Protective Activity of Methanol Leaf Extract of *Polyalthia longifolia* (Sonn.) Thw. on Ethanol-Induced Ulceration in Rats

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ABSTRACT: The study evaluates the gastro-protective activity of methanol extract of the leaves of *Polyalthia longifolia* (Sonn.) Thw. in Wistar rats. Single daily dose of the extract (200, 400 and 800 mg/kg body weight) were administered orally to the respective groups of experimental rats for 28-consecutive days. The control and standard groups received distilled water and ranitidine (10 ml/kg and 250 mg/kg body weight) respectively. At the end of the period, they were exposed to a single oral dose of 75% ethanol. After 60 minutes, the animals were sacrificed and the stomach evaluated for ulceration. There was significant decrease ($P<0.05$) in ulcer count (1.00±0.00 and 0.00±0.00) in the 400 and 800 mg/kg treated groups respectively compared to control (26.00±2.30). Similarly, there was corresponding increase in percentage of ulcer inhibition (96.2% and 100%) due to the activity of the extract. The extract of *P. longifolia* exhibited significant dose dependent gastro-protective anti-ulcer activity.

Keywords: Gastric-ulcer; *Polyalthia longifolia*; Methanol extract; Leaf; Ethanol

Introduction

The gastric mucosa is constantly exposed to potential endogenous (acid, pepsin, biliary secretion) and exogenous (*Helicobacter pylori*, excessive use of alcohol, drugs) toxic stimuli as well as stressful life habits (Harold *et al*., 2007; Laine *et al*., 2008). Gastric ulcer occurs with an imbalance between aggressive factors and innate protective factors (Izzo and Borrelli, 2000; Mohan, 2002; Dashputre and Naikwade, 2011). The condition is more prevalent in the elderly (Groenen *et al*., 2009).

Plant parts or exudates serve as major raw constituents used by several herbal industries as basis for many potent drugs and herbal products (Singh, 2015). *Polyalthia longifolia* (Sonn.) Thw., family Annonaceae, is a lofty evergreen tree native to India. It is usually planted to alleviate noise pollution (Katkar *et al*., 2010). It grows naturally and develops into tree with masses of shade. The plant serves significant role in Ayurveda. It has been reported for its forklore use to treat fever, diabetes, skin diseases, helminthiasis, hypertension and constipation. Also, the bark is employed in the treatment of digestive, circulatory and urinary systems, including pyrexia, rheumatism, scorpion sting, menorrhagia and diabetes (Katkar *et al*., 2010; Savithramma *et al*., 2011). This study was aimed at evaluating the gastro-protective property of methanol leaf extract of *P. longifolia.*

Materials and Methods

Plant material: The leaves of *P. longifolia* tree were collected from University of Benin, Benin City, Edo state, Nigeria. The specimen was authenticated in the herbarium of the Department of Plant Biology and Biotechnology (PBB), University of Benin, Benin City, Nigeria, with voucher number UBHp346.

Preparation of plant extract: The leaves were air-dried then ground to powder in an electric milling machine. Powdered sample was extracted with methanol in a tightly covered container by cold maceration at room temperature for 72 hours (with periodic stirring and shaking). The filtrate was concentrated with the aid of water bath at 99.5 ^{0}C to obtain the methanol leaf extract of *P. longifolia* (*PLLE*).
*Experimental animals***:** Male and female albino Wistar rats weighing an average of 171 g were used for the study. They were housed under favourable conditions in the Animal Unit of Department of Animal and Environmental Biology, University of Benin. The rats were grouped into five cages containing three (3) rats each. Two weeks acclimatization was allowed and they were fed with pellet diet and tap water *ad libitum*. All animals were handled in accordance with ethical guidelines of the Faculty of Life Sciences, University of Benin, Benin City, Nigeria.
Experimental design: All animal groups were maintained on repeated single daily dose treatment for 28 consecutive days. The treated groups received daily oral doses of 200, 400 and 800 mg/kg respectively of *PLLE*, while ranitidine (250 mg/kg) and distilled water (10 ml/kg) were administered orally to serve as standard and negative control respectively. After the 28-day period, all animals were fasted overnight prior to administration of 75% ethanol, then 60 minutes following ethanol exposure, the animals were sacrificed under chloroform anaesthesia. The stomach was isolated for evaluation of ulcer counts and scores (Clementi *et al*., 1998; Guedes *et al*., 2008).
Macroscopic evaluation of stomach: Each stomach was opened at the greater curvature, rinsed with normal saline and examined using ×10 magnifying lens. The numbers of ulcerations were counted, and ulcer severity determined (Clementi *et al*., 1998; Guedes *et al*., 2008). Below were the ulcer score:
0= Normal coloured stomach, 0.5= Red colouration, 1.0= Spot ulcers, 1.5= Haemorrhagic streaks, 2.0= Deep ulcer, and 3.0= Perforations.
Microscopic evaluation: Prepared slides were examined under microscope to determine the histopathologic status of the gastric mucosa.
Statistical analysis: Data obtained were subjected to statistical analyses using SPSS version 16.0 package. The values were presented as mean±SEM. One way ANOVA with Duncan post hoc test were also performed. *P-value*< 0.05 was considered to be statistically significant.

Results

Table 1 shows the ulcer severity in rats treated with 400 mg/kg *PLLE* outclassed distilled water in both ulcer severity and ulcer count, however only 800 mg/kg of the extract provided 100% inhibition, which compared favourably to the standard drug, ranitidine.

Table 1: Effects of methanolic leaf extract of *P. longifolia* on ethanol-induced stomach ulceration

Treatment	**Dosage mg/kg**	**Ulcer Count**	**Ulcer Severity Score**	**% inhibition**
Distilled water	10 ml/kg	26.00±2.30	3.00±0.00	0
Ranitidine	250	9.66±1.76^b	1.33±0.33^b	62.9
PLLE	200	14.50±0.50^a	3.00±0.00	44.2
PLLE	400	1.00±0.00^c	1.33±0.66^b	96.2
PLLE	800	0.00±0.00^d	0.00±0.00^c	100

Values are mean±SEM; n=3. Values with similar superscript within a column are not significantly different, p>0.05.
Plate 1 shows the protective effect of methanol leaf extract of *P. longifolia* on ethanol induced stomach ulcer. At 800 mg/kg the extracts exhibited a complete stomach protection in the mucosa lining wall when compared with untreated group, presenting with mucosal devitalisation, erosion of the stomach wall and ulceration.

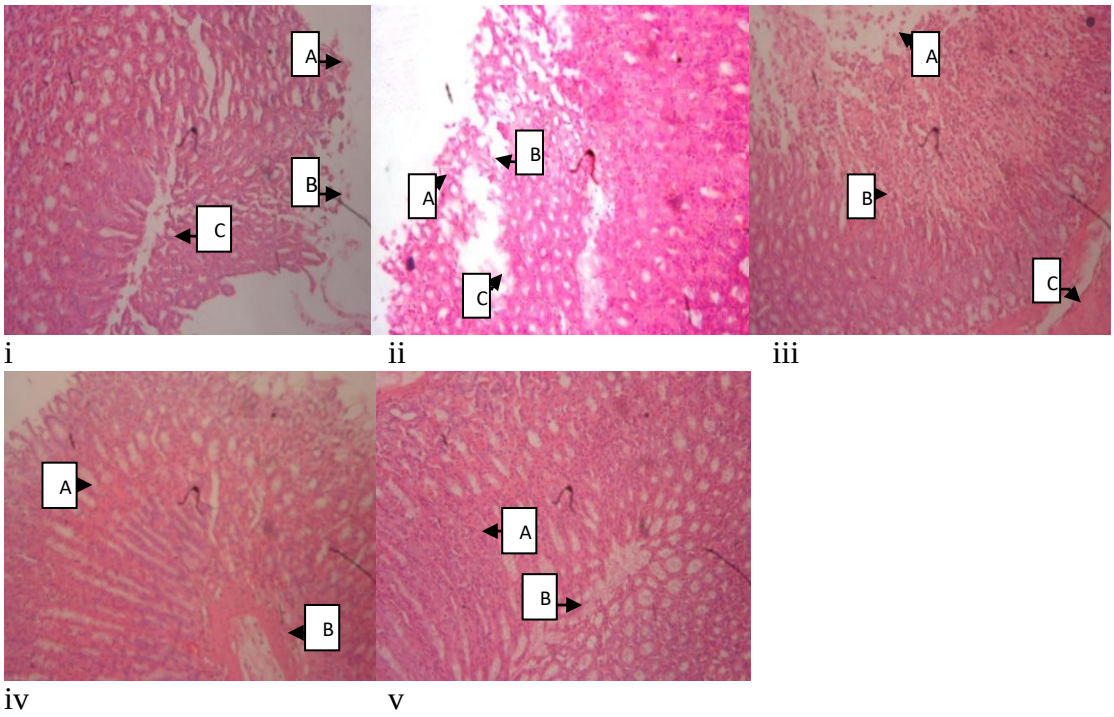

Plate 1: Protective effect of methanolic leaf extract *Polyathia longifolia* against ethanol-induced ulcer. (i). Control: (A) mucosal devitalisation and erosion, (B) ulceration (C) gastric pit. (ii). Ranitidine: (A) mild mucosal devitalisation and mild erosion (B) ulceration (C) gastric pit. (iii). 200 mg/kg *P. longifolia* extract: (A) mild focal mucosal erosion (B) normal mucosa (C) muscularis mucosa. (iv). 400 mg/kg of *P. longifolia*: (A) fairly normal mucosa (B) muscularis mucosa. (v). 800 mg/kg of *P. longifolia*: (A) normal mucosa (B) muscularis mucosa. (H & E stain; 10x).

Discussion

Under normal conditions the gastric mucosa is able to resist damage from low pH and pepsin present in the gastric lumen by a variety of defense mechanisms which include continuous layer of epithelial cells connected by tight gap junctions, which generates bicarbonate, mucus, phospholipids, peptides, prostaglandins, and heat shock proteins; In addition, there are other mechanisms such as cell renewal; continuous blood flow; production of nitric oxide and hydrogen sulfide; and sensory innervation (Tarnawski *et al*., 2013). In this study, methanol leaf extract of *P. longifolia* (*PLLE*) demonstrated gastro-protective role by significantly reducing the incidence of gastric ulcerations in a dose dependent manner. *PLLE* (400 and 800 mg/kg) provided significant ($P<0.05$) ulcer protection as indicative by ulcer counts of 1.00±0.00 and 0.00±0.00, as well as 96.2% and 100% inhibition respectively when compared with the control (26.00±2.30 ulcer count and 0% inhibition). It was also observed that 800 mg/kg of *PLLE* outclassed that of the standard drug (250 mg/kg ranitidine) in all ulcer indices evaluated in this study. This is similar to the work of Kayode *et al*. (2009). Earlier study reported that ethanolic leaf extract (300 mg/kg) of *P. longifolia* produced 53.69% ulcer inhibition (Malairajan *et al*., 2008). Histological studies also shows that the highest dose of *PLLE* maintained the integrity of the gastric mucosa by preventing the adverse effect of ethanol administration. This is unlike the untreated group and lower dose treatments, with evidence of severe to generalized erosions in the gastric epithelium. This is line with earlier report that ethanol caused massive damage to villi and crypts of the submucosa when compared with treated groups (Schneeweiss *et al*., 2006).

Pathogenesis of mucosal damage in the stomach includes generation of reactive oxygen species (ROS) that play vital role in the formation of lipid peroxides, accompanied by impairment of anti-oxidative enzyme effect of cells (Konturek *et al*., 2000). The suppressed ulcerogenic tendencies of ethanol-induced ulcer of *P. longifolia* may be an indication of antioxidant potential. Alternatively, peptic ulcer (gastric or duodenal ulcer) results from hyper activity of pepsin, and acidic environment is required for pepsin activation (Samloff, 1989). Ranitidine, a known histamine-blocker, successfully suppresses acid release, therefore decreasing pepsin activity (Black *et al*., 1972). Plant extracts may also inhibit acid-activation of pepsin, thus acting as an effective defense in the treatment of peptic ulcer disease (Schneeweiss *et al*., 2006). Ethanolic leaf extract of *P. longifolia* reportedly

produced significant reduction in acid output (Pradeepkumar *et al*., 2015). It is therefore possible that *PLLE* might be acting similarly as ranitidine by lowering acidity and depressing the activation of pepsin.

Conclusion

Methanol leaf extract of *P. longifolia* reduced gastric ulcer incidence in a dose-dependent manner when compared to control. Total anti-ulcer inhibition was possible at the peak test dose, thus indicating a high prospect of the extract as an anti-ulcer agent. The activity may be mediated through antioxidant or acid suppression mechanisms. However there is need to determine the phytoconstituents responsible for the gastro-protective anti-ulcer activity of the extract.

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