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Acute Oral Toxicity and Antiulcer Activity of *Piliostigma thonningii* Leaf Fraction in Albino rats

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ABSTRACT

Piliostigma thonningii is being used in Nigerian traditional medicine for the treatment of various diseases including gastric ulcer. The present study was undertaken to validate the anti-ulcer potential and acute oral toxicity of Piliostigma thonningii leaf. The lethal dose (LD_{50}) of the plant was found to be safe up to 3000mg/kg body weight. The antiulcer property was investigated against ethanol induced gastric ulcer in albino rats. Six groups of five adult albino rats each were orally treated as group 1(control) normal saline, group 2(standard drug) Cimetidine (100mg/kg), group 3 – 5 sequential fractions (n-hexane, ethyl acetate, methanol and aqueous) of Piliostigma thonningii leaf (100mg/kg) respectively. The result showed all the fractions significantly (P<0.001) produce protection in the ethanol induced ulceration and reduced the ulcer index when compared the control. Preliminary phytochemical screening of the fractions revealed the presence of flavonoids, terpenoids, tannins, saponins and glycosides. In conclusion, the present study provides preliminary data on the antiulcer potential of Piliostigma thonningii leaf and support the traditional uses of the plant for the treatment of gastric ulcer.

Key words: *Piliostigma thonningii,* leaf, fractions, antiulcer, acute toxicity

INTRODUCTION

An ulcer is basically an inflamed break in the skin or mucus membrane lining the alimentary tract. Ulceration occurs when there is a disturbance of the normal equilibrium caused by either enhanced aggression or diminished mucosal resistance [1]. About 19 out of 20 peptic ulcers are duodenal while gastric ulcers found in the stomach wall are less common [2]. The gastric mucosa is continuously exposed to potentially injurious agents such as acids, pepsin, bile acids, food ingredients, bacterial products (*Helicobacter pylori*) and drugs [3]. These agents have been implicated in the pathogenesis of gastric ulcer, including enhanced gastric acid and pepsin secretion, inhibition of prostaglandin synthesis and cell proliferation growth, diminished gastric blood flow and gastric motility [3]. Symptoms of ulcer include epigastric pain of a burning or gnawing nature (postprandial pain and pain relieved by food or antacids), nausea, vomiting, belching and bloating. Complications of protracted untreated cases include anemia caused by gastro-intestinal blood loss, weight loss attributed to a reduced appetite caused by fear of pain and vomiting associated with a gastric ulcer or pyloric stenosis and mucosal perforation [4]. Current management of peptic ulcer disease involves the use of proton pump inhibitor (PPI), an antibiotic and metronidazole (triple therapy) [5]. High cost of treatment and unbearable side effects of the conventional anti-ulcer drugs leads to poor compliance and resultant treatment failures. There is, therefore, the need to develop safe, effective and affordable alternatives in the symptomatic management of peptic ulcer disease.

Piliostigma thonningii Schum is a leguminous plant that belongs to the Caesalpinioidecae family which consists of about 133 genera [6]. The plant is widely distributed in Africa and Asia, perennial in nature and its flowers which are produced around November and December are white or pink in color. It bears hairy flat-pod fruits that turn nesty-brown and woody on maturity and usually persist on the plant till between June and September [7]. Locally, the plant is known as Kalgo (Hausa), monkey bread or camel's foot. A warm infusion of the bark and leaves is used to relieve fever, toothache, wound healing, cough and chest complains [8]. The roots and twigs is used locally in the treatment of dysentery, fever, respiratory ailments, snake bites, hookworm and skin infections [7]. In northwest Nigeria, the leaves are used in the treatment of stomach ache, wound

healing and ulcer. Hence, considering the traditional claim, the present study was designed to evaluate the antiulcer activity of sequential fractions of *piliostigma thonnigii* leaf in albino rats.

MATERIALS AND METHODS

Chemicals

All chemicals used in this study were of analytical grade.

Collection of plant material

Fresh leaves of *piliostigma thonnigii* were collected in January 2012 from the wilds of Aliero local government area of Kebbi State, Nigeria and were authenticated at the Botany Unit, Department of Biological sciences, Kebbi State University of Science and Technology, Aliero.

Preparation of plant material

Fresh leaves of *Piliostigma thonningii* were air-dried at room temperature for two weeks, cut into small pieces and homogenized into coarse powder using a blender.

Hydromethanolic extraction

Ten gram of coarse powder was extracted using 75% methanol for 24 hours. Thereafter it was filtered with muslin cloth and refiltered using Whatman filter No.1. The filtrate was evaporated to dryness and used for the acute oral toxicity studies and preliminary phytochemical analysis.

Sequential extraction

Sequential extraction with solvents of increasing polarity (hexane, ethyl acetate, methanol and water) was carried out using the method described by Bruneton [9] with slight modification. Two hundred grams of coarse powder was exhaustively extracted with n- hexane for 24 hours. The combined hexane extracts were filtered and evaporated at 45^0C to yield 9.9g (4.5%). The residue obtained from filtration of the hexane extract was then extracted (24 hours) with ethyl acetate and the combined extracts were filtered and evaporated to yield 14.4 g (7.2%). The residue obtained from filtration of the ethyl acetate extract was then extracted (24 hours) with methanol and the combined extracts filtered and evaporated to yield 10.4 g (5.2%). The water fraction was prepared in the same way as the methanol and ethyl acetate extract. The yield in this case was 16.8g (8.4%). These fractions were diluted with normal saline for pharmacological studies.

Experimental animals

Albino Wistar rats (150 – 200g) of either sex were obtained from the Animal House, Nigerian Institute of Trypanosomiasis Research, Kaduna State, Nigeria. All animals were maintained under standard laboratory conditions in an animal house, fed with commercial pellet diet and water *ad libitum*. All animals were acclimatized to the laboratory environment for at least two weeks before the commencement of experiment.

Preliminary Phytochemical Screening

Phytochemical analysis was performed using standard procedures to identify chemical constituents as described by Trease and Evans [10], Harbone [11] and Sofowora [12].

Acute toxicity LD_{50} determination

The acute oral toxicity of *piliostigma thonnigii* leaves extract was performed on albino rats the animals were maintained under a standard condition. And the animals fasted overnight prior to the experiment. The behavioral changes and other changes observed in animals were recorded according to Organization for Economic and Cultural Development (OECO) 425 guideline as described by Dixon [13].

Antiulcer activity

The antiulcer activity of *P. thonningii* fractions were evaluated using ethanol induced ulceration model [14]. The rats were fasted for 48 hours but allowed free access to water *ad libitum*. They were randomly selected and divided into six groups of 5 rats each. Group I (control) received normal saline 10ml/kg body weight while group II received the standard drug (Cimetidine 100mg/kg p.o.). Groups III, IV, V and VI received 100mg/kg n- hexane, ethyl acetate, methanol and water fractions of *Piliostigma thonningii* respectively. Thirty minutes later, ulceration was induced by gastric instillation of 1ml of 99% absolute ethanol and one hour after ethanol administration, rats were anaesthetized using chloroform and the stomach were removed, opened along the greater curvature to macroscopically examine any ulcerative lesions (elongated black-red lines

parallel to the long axis of the stomach). The number, length and severity of ulcers were noted and scored on an arbitrary 0 – 3 point scale [15]. The scores were as below;

0 = Normal colored stomach.
0.5 = Red coloration.
1 = Spot ulcers.
1.5 = Hemorrhagic streak.
2 = Ulcers.
3 = Perforation.

Mean ulcer score for each animal was expressed as ulcer index. The percentage of ulcer inhibition was determined as follows;

$$\% \text{ Inhibition of Ulcer Index} = \frac{\text{mean ulcer index (control group)) – mean ulcer index (test group)}}{\text{(mean ulcer index (control group))}} \times 100$$

Statistical analysis

The data were expressed as mean ± SEM while ulcer inhibition was expressed as a percentage. The significance of the difference among the groups was assessed using one way analysis of variance followed by Dunnett's multiple comparison tests. P values less than 0.01 were considered significance.

RESULTS AND DISCUSSION

Acute toxicity study carried out on the hydromethanolic extract of *P. thonningii* leaves up to the dose of 5000 mg/kg demonstrated that the extract did not produce signs of toxicity such as behavioral changes, mortality, gross and histopathological changes of the internal organs after 14 days of observation. Hence, the oral LD_{50} of *P. thonningii* leaves in rat were estimated to be > 5000mg/kg. Preliminary qualitative phytochemical analysis of hydromethanolic extract and sequential fractions of *P. thonningii* leaves revealed the presence of tannins, saponins, terpenoids, glycosides, flavonoids and alkaloids respectively (Table 1).

The pathogenesis of ulcer remains controversial but its cause is known to be aggravated by an imbalance between the aggressive factors (i.e. acids, pepsin and *H.pylori*) and factors that maintain the mucosal integrity (i.e. mucus, bicarbonate and prostaglandin) [8]. Ethanol induced damage to gastric mucosa is associated is caused by the direct toxic effect of ethanol through reduction in mucus production, gastric mucosal blood flow, bicarbonate secretion, endogenous glutathione, prostaglandin and the release of histamine, influx of calcium, generation of free radicals and luekotriene production [16]. In the present study, *P. thonningii* leaves was found to possess a remarkable and significant ($P<0.01$) ulcer inhibition properties in all the fraction treated groups respectively compared to the control (Table 2).

Table 1: **Phytochemical analysis of *Piliostigma thonningii* leaves.**

Phytochemicals	Hydro methanolic crude extract	Hexane fraction	Ethyl acetate fraction	Methanol fraction	Water fraction
Tannins	+++	+++	+++	+++	+++
Saponins	+	+	+	+	+
Steroids	++	-	-	-	-
Terpenoids	+++	+	+	+	+
Glycosides	+++	+++	+++	+++	+++
Phlobatannins	-	-	-	-	-
Anthraquinones	-	-	-	-	-
Cardenolides	++	-	-	-	-
Flavonoids	+	++	++	++	++
Alkaloids	++	++	++	++	++

+ = slightly Present, **++** = moderately Present, +++ =highly present, - = Not detected.

Table 2: Effects of sequential fractions of *Piliostigma thonningii* on ethanol induced gastric ulceration in albino rats

Group	Treatments	Dose	Ulcer Index	% Ulcer Inhibition
I	Ethanol (control)	1ml	5.0 ± 0.84	_
II	Cimetidine (standard drug)	100mg/kg	1.38 ± 0.52**	72.40
III	Ethyl acetate fraction	100mg/kg	1.44 ± 0.44**	71.20
IV	Methanol fraction	100mg/kg	1.31 ± 0.49**	73.40
V	Aqueous fraction	100mg/kg	1.31 ± 0.49**	73.40
VI	n-hexane fraction	100mg/kg	1.38 ± 0.30**	72.40

Values are expressed as mean ± SEM. ** p <0.01 significantly different when compared to the control.

It is plausible to suggest that the observed antiulcer activity is associated with *P. thonningii* ability to antagonize these aggressive factors while augmenting the defensive mucosal factors that protect the gastric mucosa from injury [17]. Cimetidine, the standard drug produced 72.40% inhibition of ulcer which was not significantly different from the fraction treated groups. Cimetidine belongs to the class of H2-receptors antagonists commonly used in the treatment of peptic ulcer and gastro-oesophageal reflux disease.

Active principles such as flavonoids, tannins and terpenoids have been reported to possess antiulcer property [1]. Tannins are known to 'tar' the outer most layer of the gastric mucosa rendering it less permeable and more resistant to chemical and mechanical injury or irritant [18]. Flavonoids are polyphenolic compounds with known antioxidant properties in addition to strengthening the mucosal defense system through stimulation of gastric mucus secretion [19].

In conclusion, the present study provided preliminary data that the sequential fraction of *P. thonningii* possesses significant anti-ulcer activity in animal models. It has a gastric antisecretory and acid neutralizing effect that are comparable to reference drug Cimetidine. Further studies are required to confirm the exact mechanism underlining the ulcer healing and protecting property of this plant.

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