Gastroprotective potentials of aqueous leaf extracts of\textit{ Phyllanthus amarus} on ibuprofen-induced ulcer in Wistar rats.


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Abstract

This study investigated the gastroprotective potential of aqueous leave extracts of \textit{Phyllanthus amarus} against ibuprofen, a non-steroidal anti-inflammatory drug (NSAID) induced gastric mucosal injury in wistar rats. Male and female adult rats of average weight 200g were divided into five groups for the protective treatment. The rats were pre-treated with distilled water, omeprazole (20mg/kg as reference drug) and 250mg/kgbw and 500mg/kgbw extracts of \textit{P. amarus} leaves. After 7days NSAID (Ibuprofen (20mg/kg) was orally administered to induce ulcer. Evaluation of the untreated (Ulcer group) rats showed severe mucosal damage. The ulcer surface area and ulcer index of experimental rats administered with \textit{P. amarus} were significantly ($p \leq 0.05$) decreased. There was a significant ($p \leq 0.05$) increase in the mucus weight and percentage inhibition of rats pre-treated with the extracts compared with the untreated group. The values of the enzymatic antioxidant and oxidative stress marker, superoxide dismutase (SOD) was significantly ($p \leq 0.05$) increased while the lipid peroxidation (MDA) was decreased. Histopathological assessment of the stomach of experimental rats showed restoration of the mucosal fold suggesting the cytoprotective effect of \textit{P. amarus} as an anti-ulcer agent. This property may be attributed to the combined action of the various antioxidant components of secondary metabolites like alkaloids, phenolic acids, glycosides, flavonoids, saponins and tannins present in the leaves extract.

Keywords: Gastroprotective, \textit{Phyllanthus amarus}, Ibuprofen, Omeprazole Gastric mucosa.

Introduction

\textit{Phyllanthus amarus} (PA) is a herb of about 2cm-30cm in height that is widely found in most tropical and subtropical country. The plant has approximately 550 to 750 species which are subdivided into 10 to 17 subgenera (Calixto et al., 1998). It is a medicinal plant of interest that is rich in antioxidants and has been widely used by traditional medicine practitioners for the treatment of a wide variety of ailments (Grieve, 2008; Calixto et al., 1998). \textit{Phyllanthus amarus} has long been used for the treatment of the liver (Kamble et al., 2008) and active components of the plant have shown hepatoprotective properties (Padma and Sethy, 1999) and employed in Ayurvedic formation (Ratna, 2002; Jain et al., 2009). It exhibit anti-viral properties and used as a remedy for hepatitis B virus infection (Eldeen et al., 2010). The leaves are used as expectorant, diaphoretic, laxative diuretic (Kiritikar and Basu., 2001). The phenolic constituent alleviated the effect of anti-mycin A-induced apoptotic cascade (Guha et al., 2010). The gastroprotective activity effect of other \textit{phallantus} species was studied by Shokunbi and Odetola (2008), the acute toxicity study (Pingale and Shewale., 2011) and its ability to lower blood pressure had been reported (Siridya and Perival., 1995; Amonkan et al., 2013; Amaechina and Omoiybai., 2007).
Gastric and duodenal ulcers are the most prevalent gastrointestinal disorders (Somasundaram, 2013). Ulcer is primarily caused by an imbalance between the endogenous aggressive and protective factors in the stomach (Paguigan et al., 2014). These protective factors induce acid-pepsin secretion, mucus secretion, reduction of gastric mucosal blood flow, integrity of the mucosal barrier, impairment of cellular regeneration, suspension of prostaglandin synthesis, and growth factors (Freitas et al., 2008). Research have shown that the aggressive factors involves the continuous use of non-steroidal anti-inflammatory drugs (NSAID) such as ibuprofen, aspirin, indomethacin, naproxen, piroxicam, fenoprofen, salsalate, exaprozin etc, cause the erosion and injury of the gastric mucosal epithelia (Wallace., 2000).

**Materials and Methods**

**Plant material**

Fresh leaves of *Phyllantus amarus* [PA] were obtained from a bush farm in Kaiama town in Kolokuma/Opokuma Local Govt. Area of Bayelsa State, Nigeria. The plants were identified and authenticated by Dr. Nwosu of the Plant Science and Biotechnology Dept. of the University of Port Harcourt, Choba, Port Harcourt, Rivers State, Nigeria.

**Extract Preparation**

Freshly harvested leaves *P.amarus* (PA) was sun dried and coarsely powdered with a blender. 100gm of the powdered leaves were boiled in 500ml of distilled water for 15 minutes. The decoction was taken and allowed to cool for 45minutes at room temperature. It was then filtered twice using Whatman No1 filter paper, then evaporated to dryness in an oven at 50°C, to produce 38.13g of the aqueous extract. Stock solution of the concentrated plant samples were constituted with distilled water at 250mg/ml and different doses (250 and 500mg/kg).

**Experimental animals**

For this study wistar rats of both sex weighing between 150-200g body weights were purchased from animal house of the Department of Biochemistry, Federal University of Agriculture, Abeokuta (FUNAAB), Ogun State, Nigeria. The rats were randomly divided into five (5) groups of 4 rats per group, kept in metal cages at room temperature and allowed to acclimatize for one week under standard environmental conditions. The rats were fed with balanced rodent pellet diet and allowed free access to water *ad libitum*.

**Acute toxicity test**

Preliminary acute toxicity studies of *P.amarus* leave extract were performed on the rats in accordance to the OECD guideline no 423 (Acute Toxic Class Method) to determine the safe dose concentration of the extract to be administered. The rats were fasted for 24 hours prior to dosing with the leave extract in increasing dose up to 5000mg/kg body weight. The rats were observed at an interval of 4 hours daily for 3 days for any clinical or toxicological manifestation. It was observed that at this dose no abnormal behavior was recorded, hence 1/10th (500mg/kg) of this dose was selected for the study.

**Ibuprofen induced gastric mucosal injury studies**

In this study the rats were divided into five groups of four rats each.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1.</td>
<td>Normal</td>
<td>rats administered with distilled water only</td>
</tr>
<tr>
<td>A2.</td>
<td>Negative Control</td>
<td>rats induced with 20mg/kg ibuprofen only (Ulcer control)</td>
</tr>
<tr>
<td>A3.</td>
<td>Positive Control</td>
<td>rats induced with 20mg/kg ibuprofen + 20mg/kg Omeprazole (NSAID)</td>
</tr>
<tr>
<td>A4.</td>
<td>Pre – Treated Rats</td>
<td>rats induced with 20mg/kg ibuprofen + 250mg/kg aqueous extract of <em>P.amarus</em>.</td>
</tr>
<tr>
<td>A5.</td>
<td>Pre – Treated Rats</td>
<td>rats induced with 20mg/kg ibuprofen+ 500mg/kg aqueous extract of <em>P.amarus</em>.</td>
</tr>
</tbody>
</table>
Estimation of gross gastric lesion

Gastric mucosa ulcer appears as dark hemorrhagic lesions of the stomach. The stomach of the experimental rats was dissected along the greater curvature and examined for damage. Each damage was measured using a planimeter (10 x10 mm$^2$ = ulcer area) by viewing under microscope magnification (1.8X). The length and breadth of the ulcer area was evaluated by counting the number of small squares (2mm x2mm) of the stomach. The total number of these lesions multiplied by 4 x 1.8= ulcer area (UA mm$^2$) (Mahmood et al., 2011).

The gastric lesions were counted and ulcer index (UI) was calculated using the expression (Singh., 1999);

$$\text{UI} = (n + \text{lesion I}) + (n + \text{lesion II}) + (n + \text{lesion III})$$

Where;

I = Presence of edema, hyperaemia and single submucosal, puntiform hemorrhage (petechiae);
II = Presence of submucosal, hemorrhage lesions with small erosions;
III = Presence of deep ulcer with erosions and invasive lesions (Szelenyl and Thiemer, 1978).
$n$ = Number of ulcer.

The percentage ulcerated surface was calculated as the total area covered by all lesions expressed as a proportion of the total corpus mucosa surface area. The percentage of inhibition (%) was calculated as described by Nguelefack et al., (2005) and Ateufack et al., (2006) using the formula;

$$\text{Percentage ulcer inhibition} = \frac{\text{USc} - \text{Ust}}{\text{USt}} \times 100$$

Where;

USc = Ulcer surface area of control
USt = Ulcer surface area of treated

Statistical Analysis

All values were reported as mean ± standard deviation (SD). The statistical significance of differences between groups was assessed using one-way analysis of variance (ANOVA), followed by Dunnett’s test. A value of $P \leq 0.05$ was considered significant difference between the groups. Statistical computations were calculated using Statistical Package for Social Sciences (SPSS) Version 20,(SPSS Inc, Chicago, IL, USA).

Results

Qualitative and quantitative phytochemical composition of *P.amarus* extract.

Results of the preliminary phytochemical study of *P.amarus* extract showed the presence of secondary metabolites like alkaloids, flavonoids, glycosides, phenolic acid, saponins and tannins, (Table 1). Tables 2 and 3 shows the quantitative analysis of the phenolic acid and alkaloid content of *P.amarus* leaf extract respectively.

**Table 1. Qualitative Phytochemical analysis of *P. amarus* leaf extracts.**

<table>
<thead>
<tr>
<th>S/No.</th>
<th>Constituents</th>
<th>Aqueous leave extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Phenolic acid</td>
<td>+</td>
</tr>
<tr>
<td>2.</td>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>3.</td>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>4.</td>
<td>Glycosides</td>
<td>+</td>
</tr>
<tr>
<td>5.</td>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>6.</td>
<td>Saponins</td>
<td>+</td>
</tr>
</tbody>
</table>

(+) Presence of phytoconstituent in *P.amatus* leaf extract.
Effect of aqueous leave extract of *P.amarus* on ibuprofen induced ulcer.

The aqueous *P.amarus* extract (P.A.E) showed significant anti-ulcer effect against ibuprofen induced ulcer in a dose dependent manner. Mean ulcer area of the ulcer (negative) control group was 120.13 ± 0.41, pre-treatment with *P.amarus* extract at a dose of 250 and 500mg/kgbw significantly (p ≤0.05) reduced this to 65.87 ± 0.61 and 60.43± 0.37 respectively, while the reference drug, omeprazole treated group was reduced to 50.53± 0.40. The ulcer index result showed that the ulcer group recorded 5.83±0.05 while the omeprazole group, 250mg/kgbw and 500mg/kgbw of *P.amarus* extract was reduced (p ≤ 0.05) to 2.10± 0.10, 3.03±0.05 and 2.71±0.80 respectively (Table 4).
The percentage inhibition and mucus weight as shown in Table 5 indicate that the negative control group was reduced to 12.2 ± 0.56%, which was increased to 75.2 ± 0.16% for the omeprazole group while the rats administered with extracts of P. amarus recorded 65.3 ± 0.70% and 70.2 ± 0.30% for 250mg/kgbw and 500mg/kgbw dose respectively. The mucus weight was significantly (p ≤ 0.05) increased to 144.5 ± 2.28mg for the reference drug, omeprazole from 56.80 ± 1.47mg recorded for the ulcer control group. The values recorded for the mucus weight of rats pre-treated with P. amarus extract showed no difference with the normal control and the reference drug group rats.

**Effect of P. amarus extract on some oxidative stress and enzyme marker.**

The level of the lipid peroxidation product MDA was elevated in the ulcer control group by the ulcerogenic agent, ibuprofen (40.30 ± 0.30μmol/mg protein, p ≤ 0.05) compared to the normal control (10.53 ± 0.15μmol/mg protein) (Table 6). The MDA value in rats pre-treated with PAE and the reference drug (omeprazole) were significantly (p ≤ 0.05) reduced to 13.6 ± 0.20μmol/mg protein, (500mg/kgbw) and 13.00 ± 0.11μmol/mg protein for omeprazole group. In contrast to the results of MDA, the activity level of superoxide dismutase (SOD) was significantly (p ≤ 0.05) lower in the ulcer control group (15.10±0.15μmol/mg protein). The administration of PAE increased the SOD level 33.10±0.45μmol/mg protein at dose 500mg/kgbw and 35.60±1.34μmol/mg protein for omeprazole group (Table 6).

### Table 4: Effect of P. amarus extract on Ibuprofen induced Ulcer on ulcer area and ulcer index.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Ulcer Area (mm²)</th>
<th>Ulcer Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal control</td>
<td>0.00 ± 0.00 b</td>
<td>0.00 ± 0.00 b</td>
</tr>
<tr>
<td>2</td>
<td>Ulcer control</td>
<td>120.13 ± 0.41 a</td>
<td>5.83 ± 0.05 a</td>
</tr>
<tr>
<td>3</td>
<td>Omeprazole</td>
<td>50.53 ± 0.40 a,b</td>
<td>2.10 ± 0.10 a,b</td>
</tr>
<tr>
<td>4</td>
<td>P.A.E 250mg/kg bw</td>
<td>65.87 ± 0.61 a,b</td>
<td>3.03 ± 0.05 a,b</td>
</tr>
<tr>
<td>5</td>
<td>P.A.E 500mg/kg bw</td>
<td>60.43 ± 0.37 a,b</td>
<td>2.70 ± 0.80 a,b</td>
</tr>
</tbody>
</table>

Values are mean ± S.D of triplicate determination. Values with the same column with superscripts alphabets ‘a’ and ‘b’ are significantly different at P ≤ 0.05 when group 1 and group 2 are compared with other groups respectively.

### Table 5: Effect of P. amarus extracts on Ibuprofen induced Ulcer on % Inhibition and mucus weight

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>% Inhibition</th>
<th>Mucus weight(mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal control</td>
<td>0.00 ± 0.00 b</td>
<td>124.36 ± 3.68 b</td>
</tr>
<tr>
<td>2</td>
<td>Ulcer control</td>
<td>12.2 ± 0.56 a</td>
<td>56.80 ± 1.47 a</td>
</tr>
<tr>
<td>3</td>
<td>Omeprazole</td>
<td>75.20 ± 0.16 a,b</td>
<td>144.50 ± 2.28 a,b</td>
</tr>
<tr>
<td>4</td>
<td>P.A.E 250mg/kg bw</td>
<td>65.30 ± 0.70 a,b</td>
<td>120.60 ± 1.24 a,b</td>
</tr>
<tr>
<td>5</td>
<td>P.A.E 500mg/kg bw</td>
<td>70.20 ± 0.30 a,b</td>
<td>145.50 ± 2.24 a,b</td>
</tr>
</tbody>
</table>

Values are mean ± S.D of triplicate determination. Values with the same column with superscripts alphabets ‘a’ and ‘b’ are significantly different at P ≤ 0.05 when group 1 and group 2 are compared with other groups respectively.

### Table 6: Effect of P. amarus extract on enzymatic antioxidant

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Superoxide Dismutase (SOD) (μmol/mg protein)</th>
<th>Lipid Peroxidation (MDA) (μmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal control</td>
<td>39.20 ± 0.92 b</td>
<td>10.53 ± 0.15 b</td>
</tr>
<tr>
<td>2</td>
<td>Ulcer control</td>
<td>15.10 ± 0.15 a</td>
<td>40.30 ± 0.30 a</td>
</tr>
<tr>
<td>3</td>
<td>Omeprazole</td>
<td>35.60 ± 1.34 a,b</td>
<td>13.10 ± 0.11 a,b</td>
</tr>
<tr>
<td>4</td>
<td>P.A.E 250mg/kg bw</td>
<td>30.20 ± 0.32 a,b</td>
<td>16.50 ± 0.40 a,b</td>
</tr>
<tr>
<td>5</td>
<td>P.A.E 500mg/kg bw</td>
<td>33.10 ± 0.45 a,b</td>
<td>13.60 ± 0.20 a,b</td>
</tr>
</tbody>
</table>

Values are mean ± S.D of triplicate determination. Values with the same column with superscripts alphabets ‘a’ and ‘b’ are significantly different at P ≤ 0.05 when group 1 and group 2 are compared with other groups respectively.
Histopathology

**Figure 1:** Normal group rats. No injuries to the gastric mucosa are seen. X200

**Figure 1a:** Normal group rats showed normal gastric mucosa with normal glands, Nucleus appears distinct. X100

**Figure 2:** Untreated group rats (ulcer control). Severe injuries are seen in the gastric mucosa. NSAID (Ibuprofen) and absolute ethanol produced extensive visible hemorrhagic necrosis of gastric mucosa. X200

**Figure 2a:** Untreated ulcer group showed mucosal ulceration with sub mucosal edema, inflammation and polymorphonuclear infiltrate at the ulcer site as well as in the oedematous submucosa. X100
Discussion

The stomach is exposed to various aggressive and damaging agents like the prolong intake of non-steroidal anti-inflammatory drugs (Ibuprofen etc), absolute ethanol, smoking, HCl, bile acid etc, which induces gastric mucosa lesions causing erosion, ulceration, hemorrhage and loss of epithelial cells (Shin et al. 2013). These features were evident in this study as observed by the action of the ulcerogenic agent, ibuprofen in the ulcer control group. To maintain the integrity of the stomach, gastric mucosa damage is usually treated with antacids, proton pump inhibitors, histamine H2 receptor type, prostaglandins, cytoprotective agents, gastric antisecretory agents and antibiotics to reduce gastric acid secretion, increasing the production of gastric mucus, increase bicarbonate production and providing defense mechanism to protect the surface epithelial cells (Ateufack et al. 2015). In this study, pre-treatment with increasing dose of P.amarus extract demonstrated cytoprotective tendency to the gastric mucosa, due to increased mucus weight accompanied by a proportional increase in proteins, this agrees with the study of Thirunavukkarasu et al., (2009). The gastro protective effect of P.amarus may be attributed to the various secondary metabolites present in the plant. Phenolic compounds are known to stimulate the production of prostaglandins based on their action as co substrate for the peroxidase reaction (Alanko et al., 1999). The action of phenols, alkaloids and tannins contribute in precipitating microproteins on the ulcer site forming a thick layer to cover the surface of the epithelial cells thereby hindering gut secretions and protect the mucosa from damage. The following active components, chlorogenic, gallic and ellagic acids of phenols as well as phyllantene and phyllantidine of alkaloids are characterized by their astringent and antimicrobial action to exhibit cytoprotective and anti-ulcer activity as reported in other plants by Gonzales et al.,(2000); Konig et al.,(1994); and Ramirez and Roa. (2003). Since, P.amarus have also shown similar cytoprotective and anti-ulcer activity, this property can be attributed to the actions of these secondary metabolites present in the plant. This is consistent with the findings on Phyllanthus niruri by Abdulla et al., (2010); Acanthopanax trifoliatus by Roslida et al., (2010). Histopathological examinations of tissues of the stomach showed flattening of the mucosal folds indicating gastroprotective effect of the plant extract. This causes a relaxation of the circular muscles and increases the production of prostaglandin (Takeuchi and Nobuhara, 1985).

The level of lipid peroxidation (MDA) was increased while the enzymatic antioxidant defense component like superoxide dismutase (SOD) was decreased due to the effect of ibuprofen. Pre-treatment with aqueous extracts of P.amarus reduced the MDA level and in contrast elevated the level SOD activity. Superoxide dismutase is an important antioxidant that converts singlet oxygen, reactive oxygen species (ROS) and free radicals to hydrogen peroxide and molecular oxygen, thus protecting tissues and cells from necrotic effect of ibuprofen (Devi et al, 2007).
Conclusion

The results from this study revealed that *P. amarus* possess gastroprotective and anti-ulcer potentials. Such protection was shown to be dose dependent as shown by the reduction of ulcer area and ulcer index and a simultaneous increase in the mucus weight. The cytoprotective effect of aqueous *P. amarus* extract may be due to the action of bioactive phytochemical components present in the plant extract which results in increasing the antioxidant activity and reducing the effect of the aggressive agent.

References


