Antiulcerogenic effects of aqueous - methanol leaf extract of *Vernonia amygdalina* on aspirin-induced gastric ulcer in male albino rats

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ABSTRACT

Currently, there is increasing trend in the use of herb for the treatment of diseases and ailments. *Vernonia amygdalina* is a valuable shrub that is widely spread in the East and West Africa and its medicinal value has been reported in tradomedicine. The effects of aqueous - methanol leaf extract of *V. amygdalina* on aspirin-induced gastric ulcer in male albino rats was investigated using standard methods. A total of 43 rats were used for the study. Thirteen rats were used for the acute toxicity study while thirty rats randomly divided into six groups (n=5) were used for anti-ulcer study. The result of the phytochemical screening revealed the presence of alkaloids, saponins, terpenoids, flavonoids, tannins, resins and steroids. The result of the acute toxicity study revealed that the extract might have LD₅₀ above 5000 mg/kg b.w. The results of the gastrointestinal pH indicated insignificant (p>0.05) increase in all groups treated with the graded doses of the extract while the groups treated with omeprazole showed significant (p<0.05) increase in pH when compared to the negative control group. Ulcer index of the groups treated with omeprazole and graded dose of the extract showed a significant (p<0.05) dose response decrease when compared to the negative control group. The result of the anti-oxidant assay showed significant (p<0.05) increase in the catalase activity of the group treated with omeprazole and the group treated with 600mg/kg of the extract but showed no significance (p<0.05) increase in the enzyme activities at lower doses of the extract when compared to the normal group. The study has shown that the extract elicited ulcer curative efficacies and perhaps justifies its use in traditional medicine for ulcer management and cure. 

Keywords: *Vernonia amygdalina*, phytochemicals, peptic ulcer, aspirin, omeprazole

INTRODUCTION

Peptic ulcer remains one of the gastrointestinal disorders that have affected many people worldwide over the centuries with over 5-10% of the world population generally affected [1]. It is a chronic disease that occurs when there is a fracture in the mucosal lining of the stomach. When a physiological balance exists between aggressive factors and mucosa defense, this balance is compromised in favour of aggressive factors which lead to injury in the gastric mucosa [2]. Some of the aggressive factors are stressful lifestyle, smoking, socio-economic status, *Helicobacter pylori*, steroidal and non-steroidal anti-inflammatory drugs, ethanol and family history [3,4]. Drugs have a devastating effect on gastric mucosa as a result they has been used to induce gastric ulcer in animal models. Report has shown that drugs compromise the integrity of gastric mucosa by aiding acid reflux into the subluminal layer of the mucosa and submucosa [5]. It may also act through a general mechanism affecting the release of hormones and the regulation of nerve function involved in acid secretion [6]. Approximately 500,000 new cases of ulcer are reported annually, with 5 million cases in the United States alone [7]. Although, ulcer is not a deadly disease, it can lead to more serious complications like gastrointestinal bleeding,
perforations, penetration of ulcer into adjacent organs and gastric outlet obstruction [8]. Several orthodox drugs have been manufactured to combat this disorder. Most of these drugs are costly and have intolerable side effects when taken. Some orthodox drugs employed in the treatment of this disorder include histamine (H₂) receptor antagonists, proton pump inhibitors, cytoprotectants, antacids, and prostaglandin analogues [1]. Medications are used to relieve the pain, heal ulcerations and delay recurrence of ulcerations. These include antibiotics, antacids and proton pump inhibitors [8]. Several drugs are available in the market for gastric ulcer therapy; however, most of these drugs are costly and associated with unwanted side effects [1]. To this effect, there is need to find alternative solutions to this global life threatening problem. In this context, this research aims to investigate the antiulcerogenic potential of the aqueous - methanol leaf extract of Vernonia amygdalina in animal models following aspirin-induced gastric ulcers to add to current knowledge on alternative treatment of gastric ulcers using plant extracts. Several prospective medicinal plants for peptic ulcer treatment have been studied and reported in literature globally [9,10,11,12,13,14,15] reported on some medicinal plants viz; Allophylus serratus Kurz, Cissus quadrangularis, Ocimum sanctum Linn, Mangifera indica L., Zingiber officinale Roscoe, Butea frondosa Roxb. L., Glycyrrhiza glabra L., Solanum nigrum L., and Terminalia chebula Retz. Similarly, medicinal plants such as Cynodon dactylon, Azadirachta indica, Glycyrrhiza glabra, Swietenia mahagoni, Bauhinia purpurea L., Ficus religiosa, Melastoma malabathricum, Ocimum sanctum, Spondias mombin L., Eruca sativa, and Osyris quadripartite have been reported as possessing some form of antulcer activity upon investigation in antulcer testing models [16,17,18,19,20,21,22,23]. A triple herbal therapy comprised of Enterica, Dyspepsia decoctions, and Natural Pain Killer (NPK 500) capsules manufactured by the Centre for Plant Medicine Research (CPMR) is currently used in the management of peptic ulcer disease at the CPMR out-patient clinic in Ghana [24]. These three products contain medicinal plants such as Carapa procera, Trichilia monadelpha, Persea americana, Trema orientalis, Momordica charantia, Vernonia amygdalina, Cassia sieberiana, Citrus aurantifolia, Bidens pilosa, Morinda lucida, Maytenus senegalensis, Psidium guajava, Cnestis ferrugineua, Spondias mombin, and Latana camara. The observed activity is these plants are attributed with the presence of flavonoids, alkaloids, terpenoids, tannins, saponins, and phenolic acids. Vernonia amygdalina commonly known as “bitter leaf” is a valuable shrub that is widely spread in the East and West Africa. It is used in trandmedicine in treatment of many ailments viz; laxative, pile (hemorrhoids) and gastrointestinal troubles [25,26,27,28,29,30], relief pain and lower body temperature, diabetes [31] etc.

MATERIALS AND METHODS

EQUIPMENT: All the equipment used in this study were of laboratory working standard. REAGENTS: All the chemicals used in this research were of analytical grade, commercially available and were obtained from Merck, England; BDH, England; and Fluke chemicals, Buchs, Switzerland.

SOURCES OF EXPERIMENTAL MATERIALS

Wistar male albino rats were purchased from the Animal House of ChrisKing, Mgbakwu, Anambra State Vernonia amygdalina leaves were collected from Ogboji Ezzagu, Ebonyi State and was identified and authenticated by a taxonomist, Mr P. Ugwuozor of Department of Biotany, UNIZIK.
METHODS
PREPARATION AND EXTRACTION OF PLANT EXTRACT

The leaves were washed, cleaned and air-dried at room temperature for 3 weeks. The dried leaves were ground into powder using a manual grinding machine. Afterwards, extraction was done by cold maceration of 1000g of ground leaves in aqueous methanol for 48 hours. This was proceeded by filtration using whatman filter paper and the filtrate was concentrated/lyophilized by freeze-dried.

DRUG AND EXTRACT PREPARATION

Aspirin was used to induce ulcer while omeprazole was used as standard anti-ulcer drug. All were purchased from Joez Pharmacy; Zik Avenue Awka, Anambra State. The drugs were dissolved in 5% 5ml tween 80 and administrated in concentrations according to their body weight as recommended by [32]. The extract was dissolved in 5% 5ml; tween 80 and administered in concentrations of 200, 400, and 600mg/kg body weight according to the recommendation of [33].

TEST FOR TANNINS

Bromine water test
Exactly 1.0ml of bromine water and 1.0ml of extract was pipetted into a test tube and observed for colour change. The appearance of red coloration indicates the presence of tannins.

Acid test
Exactly 3.0ml of extract was added to 2.0ml of HCl in a test tube. The mixture was observed for colour change or formation of precipitates. The formation of red precipitate confirms the presence of tannins.

Lead Acetate Test
Exactly 3 drops of 5% lead acetate solution were added into a test tube containing 2.0ml of extract. It was then observed for presence of precipitate.

TEST FOR FLAVONOIDS

Ferric chloride test for phenolic Nucleus
Test tube containing 1.0ml of extract, 1.0ml of 10% ferric chloride was pipette. It was mixed properly and observed carefully for colour change to greenish brown or black. Exactly 5.0ml of distilled water was pipetted into a conical flask containing 1.0ml of the extract and boiled. The soluble fraction of the mixture was decanted into a test tube while hot. Then 2 drops of olive oil were added into this test tube. The mixture was gentle agitated and observed for presence of emulsion.

Frothing Test
To a conical flask containing 1.0 ml of the extract, 5.0ml of distilled water was added and then boiled. The soluble fraction of the mixture was decanted into a test tube while hot; to 1.0ml of this, 3.0ml of distilled water was added. The solution was agitated vigorously and observed for froth.

TEST FOR RESIN

Exactly 0.2ml of the extract was mixed with 20 ml of distilled water in a beaker. The formation of precipitates indicates the presence of resins.

TEST FOR STEROID

To a test tube containing 0 .5ml of the extract was added 1ml H₂SO₄. The mixture was agitated gently and the colour at the interface was observed and recorded. One, a total of nine rats were used. The rats were divided into three groups of three rats per group. Group one was administered with 10mg/kg body weight of the extract, group two was treated with
100mg/kg body weight of the extract while group three was administered with 1000mg/kg body weight of the extract. All the administrations were done by oral intubation. The animals were monitored for twenty-four hours for mortality and general behaviour. From the result of the phase one, phase two was carried out. In this phase, a total of four rats were used, and they were divided into six groups of one rat per group. Group one was treated with 2000mg/kg body weight (bt.w), group two was treated with 3000mg/kg bt.w; group three was treated with 4000mg/kg bt.w while group four was treated with 5000mg/kg bt.w of the extract.

EXPERIMENTAL DESIGN

Thirty (30) Wistar male albino rats weighing (190-245 g) were used for this study. The rats were housed in silver-coated metal cages in Department of Pharmacology, Nnamdi Azikiwe University, Agulu. The cages were cleaned on daily basis to prevent coprophagy and maintained on sterile pellet diet and water ad libitum. The animals were exposed to warm rays of the morning Sun for about 10 minutes while their cages were being clean to keep them warm and dried daily. Before the experiments were conducted, the animals were acclimatized for one week. The rats were fasted for 24 hours before the experiment but were allowed free access to water up to 2 hours before the experiment according to the method of [26]. The animals were grouped into six (6) groups of five (5) each. Group One (1) was given served as normal control, group two (2) served as negative controls, group three (3) served as positive control group while groups four (4), five (5) and six (6) were used for anti-ulcer studies.

ANTI-ULCER EFFECTS OF THE EXTRACT

Group II, III, IV, V and VI were all administered 200mg, 5ml/kg Aspirin and after two (2) hours of this pre-treatment, (5%, 5ml) tween 80 was administered to II (Ulcer-negative control), 20mg, 5ml/kg omeprazole was administered to group III (positive control) while group IV, V and VI were administered with 200, 400 and 600mg/kg body weight of the extract respectively.

ULCER EVALUATION

Ulcers of the gastric mucosa appeared as elongated-undulating bands of hemorrhagic lesions. The gastric mucosa of each rat was thus examined for damage. The ulceration was scored as follow:

<table>
<thead>
<tr>
<th>Colour</th>
<th>Ulcer Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal coloration</td>
<td>0</td>
</tr>
<tr>
<td>Red coloration</td>
<td>0.5</td>
</tr>
<tr>
<td>Spot ulcer</td>
<td>1.0</td>
</tr>
<tr>
<td>Haemorrhagic stress</td>
<td>1.5</td>
</tr>
<tr>
<td>Deep stress</td>
<td>2.0</td>
</tr>
<tr>
<td>Perforation</td>
<td>3.0</td>
</tr>
</tbody>
</table>

Then ulcer index was calculated using the formula: $U_i = (U_n + U_s + U_p) \times 10^{-1}$. $U_i$ = ulcer index, $U_n$ = average number of ulcer animal, $U_s$ = average severity of ulcer, $U_p$ = percentage of animals having ulcer.

MEASUREMENT OF GASTRIC SECRETION AND pH

The stomach of all the animals were carefully exercised while keeping the oesophagus closed at the greater curvature and the luminal contents were collected and measured using a calibrated cylinder. The gastric juice was collected and centrifuged at 300 rpm for 10 minutes and the pH measured using digital pH meter.
MEASUREMENT OF THE TOTAL ACIDITY

The total acidity of the luminal content of each animal was estimated using the formula described by [10].

$$\text{Total acidity} = \frac{0.02 \text{M NaOH} \times X}{Y}$$

where $X =$ vol. of NaOH, $Y =$ vol of total secretion obtained from the stomach

ASSAY OF ANTI-OXIDANT ENZYME (CATALASE)

The anti-oxidant, catalase test was done using the method of [5]. 0.1ml of the serum was mixed with 0.4 ml of hydrogen peroxide ($\text{H}_2\text{O}_2$) and 1 ml of PO$_4$ buffer ($\text{pH}$ 7) and was incubated for 1 minute. Then 2 ml of 5% $\text{K}_2\text{Cr}_2\text{O}_7$ was added to stop the reaction. It was mixed and boiled for 10 minutes, cooled and read at 570 nm wavelengths. One unit of activity was defined as an enzyme activity (u/ml) degrading one micromole of H$_2$O$_2$ in 1 minute under standard condition.

Statistical Analysis

Data were analyzed using the Statistical Package for Social Sciences (SPSS) version 10 and the group means were compared by Duncan’s Multiple Range Test. Differences between means was regarded significant.

RESULTS

Qualitative Phytochemical Analyses of $V$. amygdalina Leaf Extract. The results of the qualitative phytochemical screening of the aqueous - methanol leaf extract of the $V$. amygdalina are shown in Table 1.

Table 2: Phytochemical Test for the Aqueous Methanolic Leaf Extract

<table>
<thead>
<tr>
<th>S/N</th>
<th>Phytochemical constituents</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Resin</td>
<td>+</td>
</tr>
<tr>
<td>2.</td>
<td>Saponin</td>
<td>++</td>
</tr>
<tr>
<td>3.</td>
<td>Tannins</td>
<td>++</td>
</tr>
<tr>
<td>4.</td>
<td>Flavonoids</td>
<td>++</td>
</tr>
<tr>
<td>5.</td>
<td>Terpenoids</td>
<td>+</td>
</tr>
<tr>
<td>6.</td>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>7.</td>
<td>Steroids</td>
<td>++</td>
</tr>
</tbody>
</table>

Guide: - = Not present, + = Present, ++ = Fairly present, +++ = Appreciable amount

Mean lethal dose (LD$_{50}$).

Acute toxicity screening (LD50) showed that the extract had an oral LD50 > 5000mg/kg in wistar albino rats are shown in tables 3 and 4 below.

Table 3: Results of mean lethal dose (LD$_{50}$)

<table>
<thead>
<tr>
<th>Phase 1</th>
<th>Dose (mg/kg)</th>
<th>Death recorded</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>10</td>
<td>0/3</td>
</tr>
<tr>
<td>II</td>
<td>100</td>
<td>0/3</td>
</tr>
<tr>
<td>III</td>
<td>1000</td>
<td>0/3</td>
</tr>
</tbody>
</table>

LD$_{50}$ >1000mg/kg
Table 4: Results of mean lethal dose (LD$_{50}$)

<table>
<thead>
<tr>
<th>Phase II</th>
<th>Dose (mg/kg)</th>
<th>Death recorded</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>2000</td>
<td>0/1</td>
</tr>
<tr>
<td>II</td>
<td>3000</td>
<td>0/1</td>
</tr>
<tr>
<td>III</td>
<td>4000</td>
<td>0/1</td>
</tr>
<tr>
<td>IV</td>
<td>5000</td>
<td>0/1</td>
</tr>
</tbody>
</table>

LD$_{50}$ >5000mg/kg

Figure 1: Effect of aqueous–methanol leaf extract of *V. amygdalina* on PH of the Gastric contents of the Animals. Bars with different letters showed significant value at P<0.05. The results of the pH screening showed a significant increase at p<0.05 in all the treated groups compared to ulcer group (group 2). The increase is higher in group treated with omeprazole (group 3). The effects of the plant extract are dose dependent. Though, omeprazole is more potent in reducing the gastric pH of the stomach.
Figure 2: Effect of aqueous - methanol leaf extract of *V. amygdalina* on total acidity (g/l) of the luminal contents of the Animals. Bars with different letters showed significant value at P<0.05. The results of total acidity showed a significant higher contents at p<0.05 in group 2 compared to treatment groups except group 4. The decrease is more in group 2 when compared to others. The effects of the plant extract is dose dependent.

Figure 3: Effect of aqueous - methanol leaf extract of *V. amygdalina* on Ulcer Index value of the Animals. Bars with different letters showed significant value at P<0.05. The ulcer index study showed a general decrease in the treatment group; 3, 4, 5 and 6. However, the decrease is not significant (p>0.05) in group 3 and 4 but significant at p<0.05 in group 5 and 6 when compared to the negative control group after 2 hours of treatment.
Figure 4: Effect of aqueous – methanol leaf extract of *V. amygdalina* on catalase activities of the animals. Bars with different letters showed significant value at P<0.05. The result of the anti-oxidant (catalase tests) assay showed significant (p < 0.05) increase in the enzyme activities in all the treated groups when compared to group 2. However, there is no significant at p<0.05 change in group 3 when compared to group 4. The effects of the plant extract is dose dependent.

**DISCUSSION**

Ulcers are reported as the most common cause of hospitalization for upper gastrointestinal (GI) bleeding and are often a clinical concern due to the widespread use of aspirin and nonsteroidal anti-inflammatory drugs, both of which have been shown to induce ulcer. Drug consumption has been considered as one of the leading causes of ulcer in humans; hence, researchers used the animal model of gastric injury induced by aspirin to simulate conditions that humans may be exposed to study the antiulcer efficacy of natural products or new therapeutics intended to be used for gastric protection [34]. In this study, ulcer was induced with Aspirin at 200mg/kg body weight for 2 hours as shown by the ulcer index of the negative control compared to the normal. The reduction of gastric acid production as well as the protection of gastric mucosa has been the major approach for the treatment and management of peptic ulcer [35], hence the use of *V. amygdalina* as an anti-ulcer agent in this study. The phytochemical screening of the aqueous – methanol leaf extract of *V. amygdalina* revealed the presence of saponins, tannins, flavonoids, alkaloids, terpenoid, resins and phenols. This study is in conformity with the reports of [36,37] which previously reported the presence of these phytochemicals in *V. amygdalina*. The presence of these phytochemicals may be responsible for either gastric acid reduce or protection of the gastric mucosa or both. The high margin of safety was specified by the fact that in the LD50 determination, no death occurred even after an oral dose of 5000mg/kg in rats. This showed that the extract of *V. amygdalina* may have LD50 above 5000 mg/kg body weight of the extract.

In the curative studies, there was a significant increase at p<0.05 in the stomach pH in all the groups treated with graded doses of *V. amygdalina* when compared to the negative control group especially at a dose 600mg/kg body weight in dose dependent manner. Though, omeprazole is more potent in
reducing the gastric pH of the stomach. This reduction in pH is in line with the objectives of ulcer treatment and management [38]. As shown in Fig.3, oral administration of aspirin induced gross lesions in the gastric lumen of rats with markedly high ulceration index. The ulcer index study showed an extreme significant (p<0.05) decrease in the ulcer index of the stomach at 600 and 400mg/kg body weight of the aqueous methanol leaf extract of *V. amygdalina* and a slight decrease at 200mg/kg body weight when compared to the negative control group after 2 hours of treatment. This shows that the curative effect of *V. amygdalina* extract could be more potent on the protection of gastric mucosa rather than pH reduction. The report of this study is accordance with the result of [39]. Hydrolysable tannins contain glucose moiety and have been used internally as astringent and as heavy metal antidote [40]. Tannins being astringent may precipitate microproteins on the site of ulcer thereby forming an impervious protective pellicle on the lining to resist the attack of proteolytic enzymes [41]. This could be likened to the effect of drugs which act by providing a cytoprotective defense against acid peptic digestion. [42] reported the biological properties of tannins and observed that tannins have anti-cancer activity and can be use in cancer prevention, thus suggesting that *V. amygdalina* has potential as a source of important bioactive molecules for the treatment of cancer. The presence of tannins in *V. amygdalina* supports the traditional medicinal use of this plant in treatment of inflamed tissues. Studies have revealed the inhibitory effect of saponins on inflamed cells and to acts as gastro protective agents [43]. Saponins in *V. amygdalina* supported the usefulness of this plant in managing inflammation.

**CONCLUSION**

The *V. amygdalina* contained vase array of phytochemicals in variable proportion. The aqueous - methanol leaf extract of *V. amygdalina* poses anti-ulcer effect. This effect might be due to the synergic effect of these phytochemicals.
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17. Zabidi, Z., Zainulddin, WNW., Mamat, SS et al. (2012). Antiulcer activity of methanol extract of Melastoma malabathricum leaves in


