Anti-ulcerogenic activity of the powder fraction of leaves of *Aspillia africana* against ethanol-induced gastric ulcer in rats: Possible role of mucus and antioxidant effect

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Abstract

Massive alcohol drinking can lead to gastric ulcer. In the present study we investigated the gastroprotective effect of powder fraction of the leaves of *Aspillia africana* (AA) in ethanol (EtOH) induced oxidative stress and peptic ulcer in rats. The rats were divided into six treatment groups, which were the normal control group, negative control group (ethanol-induced) and three treatment groups: AA at the doses of 100 mg/kg body weight (BW), 200 mg/kg BW and 400 mg/kg BW, and the positive control group treated with famotidin at the dose of 100 mg/kg BW. Gastroprotective effect was measured by the ulcerative lesion index, ulcer surface area, percentage of lesion area. Antioxidant and histopathological analyses were also conducted to gain additional data regarding the gastroprotective effect of AA in the rats’ stomachs. AA showed protected the gastric mucosa from ethanol ulceration, as revealed by the improved macroscopic and histological appearance. AA significantly increased the gastric homogenate content of GSH and inhibited the lipid peroxidation as revealed by the reduced gastric content of malondialdehyde (MDA). AA possesses gastro-protective activity, which could be attributed to a mucus production, antioxidant, and regeneration activities.

Keywords: Gastric ulcer; *Aspillia africana*; Powder fraction; Reactive oxygen species; Histothology

1. Introduction

Gastric ulcers are one of the most common diseases of the digestive system. The pathophysiology of this disease is a multifactorial process that is caused by an imbalance of gastric mucosa-protecting (pepsin) and gastric mucosa-destroying factors (acid) and is induced by infection, smoking, stress, the prolonged use of nonsteroidal anti-inflammatory drugs (NSAIDs), and excessive alcohol ingestion [1].

Ethanol is a harmful agent associated with multiple pathologies and can be orally applied to experimental animals to create acute gastric lesions [2]. The experimental model of ethanol-induced gastric injury mimics several features of the human condition and thus provides a mean for assessing agents with potential anti-ulcer actions along with their implicated mechanisms for gastric protection [3-5].

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The type of gastric ulcer induced by ethanol tends to erode the gastric tissue, causing extreme damage to the gastric mucosa, including haemorrhagic damage to the gastric mucosal lesions and mucosal oedema [6], the release of pro-inflammatory cytokines [2, 7], and invasion of activated neutrophils, apoptosis, and oxidative stress due to overproduction of reactive oxygen species (ROS) [2, 8]. ROS are the main mediators of oxidative stress and decrease the activity of antioxidant enzymes (superoxide dismutase-SOD and catalase-CAT) and non-enzymatic antioxidant (glutathione; GSH) [9]. These are some of the causes of destruction of cell membranes and are involved in the pathogenesis of acute gastric ulcers induced by ethanol.

*A. africana* belongs to the family Asteraceae and has been used by many African communities in the treatment of a range of health conditions. The plant is used to treat inflammatory conditions as well as osteoporosis, stomachache, diarrhea, measles, malaria, tuberculosis, cough, gastric ulcers, sores, diabetes, rheumatic pains, bee, scorpion and wasp stings, ear infections, febrile headaches, and gonorrhea and is used as a contraceptive. *A. africana* is also prominently known for its wound healing properties. The plant, though often known as the hemorrhage plant or wild sunflower, is referred to by various names by different communities, such as Makayi in Luganda (Uganda), Orangila in Igbo (Nigeria), Nyana in Kissi (Sierra Leone), Fofot in Akan-akyem (Ghana), Mbalu in Kpe (Cameroon), Soumadibrouin among the Malinke (Côte d’Ivoire), and Winnih in Mano (Liberia) [10].

Moreover, a literature review demonstrated that the phytochemical of *A. africana* include flavonoids, alkaloids, tannins, saponins, terpenoids, sterols, phenolic compounds, and glycosides. Essential oils from the leaves of the plant are rich in monoterpenes, sesquiterpenes, α-pinene, and germacrene, which are important therapeutic ingredients [11]. The broad range of antimicrobial and biological activities, including anti-inflammatory, haemostatic, oxytocic, gastroprotective, antiulcer, wound healing, anticancer, antihypertensive, and antidiabetic potentials may be attributed to these groups of active therapeutic components of the plant [11].

The aim of this study was to evaluate the gastroprotective effects of powder fraction of leaves *A. africana* against ethanol-induced gastric ulcers in rats and the potential underlying mechanism.

### 2. Material and methods

#### 2.1. Chemicals and Reagents

Absolute ethanol was obtained from Sigma (Sigma-Aldrich Pty Ltd., Darmstadt, Germany). Famotidine (MilliporeSigma, Oakville, ON, Canada) was used as a reference antiulcer drug.

#### 2.2. Preparation of the powder fractions of leaves of *A. africana*

The plant material was harvested in Batie, Cameroon and identified at the National Herbarium in Yaounde-Cameroon in comparison with the existing Voucher specimen N° 6555/SRF/CAM. The powder fractions of the leaves of *A. africana* was prepared according to the methods previously described by Mbassi [12]. The leaves of *A. africana* were milled using an electric Ultra-Centrifugal Mill ZM 200 (Haan, Germany) operating at 12,000 rpm (8 049.6 g) with mesh sieve of 1 nm. The powder fractions were produced on an Analysette 3 Spartan apparatus (Fritsch, Idar-Oberstein, Germany). In this respect, 100 g of the leaves of *A. africana* powder was poured on a sieve column of decreasing sizes 315, 212, and 180 µm, ending with the collecting pan. The system was allowed to vibrate at an amplitude of 0.5 mm for 10 min generating powders of respective sizes >315, 212-315, 180-212 µm, and <180 µm. There after, the powders on the respective sieves and collecting pan were collected, packed in polyethylene bags, and stored at 10 °C until to use. The finest powder fraction was used for the rest of this study.

#### 2.3. Animals

Adult male *Wistar albinos* rats weighing 150–200 g was purchased from Laboratory of Department of life Sciences, Higher Teacher Training College, University of Bertoua. They were maintained under standard environmental conditions: light–dark cycle, 12–12 h; temperature, 25 ± 2 °C; humidity, 55–65%. The rats were allowed ad libitum access to food and water during the experimental period.

#### 2.4. Ethical Approval Statement

The experimental protocols performed in this study were designed in accordance with Good Laboratory Practice and approved by the Cameroon National Ethics committee (Reg. No FWAIRB00001954).
2.5. Experimental Procedures

The Absolute ethanol was used to induce ulcers in the gastric mucosa according to the method of Hara and Okabe [13]. Male rats were fasted for 36 h before administration of extract. The rats were divided into 6 groups, as follows: negative control or normal group (normal healthy rats that didn’t receive extract, drug, or ethanol during the research), ethanol intake rats (received only 1 ml of ethanol per rat), famotidin treatment experimented group (recipient Famotidin 100 mg/kg + 1 ml ethanol per rat), experimental group 1 (received fraction of leaves of A. africana at a dose of 100 mg/kg + 1 ml ethanol per rat), experimental group 2 (received fraction of leaves of A. africana at a dose of 200 mg/kg + 1 ml ethanol per rat), experimental group 3 (received fraction of leaves of A. africana at a dose of 400 mg/kg + 1 ml ethanol per rat). The animals were killed using ether, the abdomen of each opened and the stomachs removed. The ulcers produced in the glandular region of each stomach were measured and scored as earlier described by Tan et al. [14] and the ulcer index (UI), percentage of inhibition (%I) and percentage of ulcerated surface (%US) were calculated.

2.6. Gastroprotective Analysis

The stomach of each rat was removed and opened along the greater curvature. Then, the stomach was gently rinsed with water to remove the gastric contents, for subsequent scanning and measurement of the ulcerative lesion index (IU). Ulcerated area and ulcer index were calculated as described by Tan et al [14]. Ulcerated area: length x width. Ulcer scores were allotted as follows: no ulcer = 0.0; ulcer surface ≤0.5mm² = 1; ulcer surface >0.5 ≤ 2.5mm² = 2; ulcer surface >2.5 ≤ 5mm² = 3; ulcer surface >5 ≤10mm² = 4; ulcer surface >10 ≤15mm² = 5; ulcer surface >15 ≤ 20mm² = 6; ulcer surface >20 ≤ 25mm² = 7; ulcer surface >25 ≤ 30mm² = 8; ulcer surface >30 ≤ 35mm² = 9; and ulcer surface >35mm² =10. The ulcer index (UI) was calculated with the following formula:

\[ UI = \frac{1}{n} \sum_{i=1}^{n} \text{score} \pm \text{SEM} \]

The percentage of ulcerated area in relation to the total stomach was calculated using the following equation:

\[ \% \text{Ulcerated area} = \frac{\text{total ulcerated area}}{\text{total stomac area}} \times 100 \]

The percentage of inhibition was calculated using the following equation:

\[ \text{Inhibition (\%)} = \frac{\text{Ulcer index control group} - \text{Ulcer index test group}}{\text{Ulcer index control group}} \times 100 \]

2.7. Histopathological Examination

Stomach tissues from each group were fixed in 10% formalin solution for 24 h, dehydrated, and embedded in paraffin. Tissue sections (5 µm) were cut and stained with hematoxylin and eosin (H&E) solution. Histopathological changes in the stomach, including edema, blood vessel changes, white blood cell infiltration, and glandular damage, were analyzed under a light microscope at 400× magnification.

2.8. Measurement of Oxidative Indicators

After photographing, oxidative stress parameters were measured in gastric tissue samples. Superoxide dismutase (SOD) activity was measured using a standard method [15] and expressed in mmol/g of protein. Catalase (CAT) was determined [16, 17] and expressed as µmol of H₂O₂/min/mg of protein. Reduced glutathione (GSH) was measured based on the reaction between 2,2-dithio-5,5-dibenzoic acid and the thiol (SH) groups of glutathione to yield a complex whose absorbance was read at 412 nm [18, 19] and expressed as U/mg of protein. Lipid peroxidation was assessed by measuring malondialdehyde levels in gastric tissue samples (MDA) [20], expressed as pmol /mg of wet stomach tissue. Tissue protein was measured using the Biuret method of protein assay [21].

2.9. Statistical Analysis

Data were presented as mean ±SEM. The data were analyzed by one way analysis of variance (ANOVA) followed by Dunnett’s post-hoc test using Graphpad Prism 9.0 Software. Values of P<0.5 were considered as statistically significant.
3. Results

The gastroprotective effect of powder fraction of leaves of *A. africana* was measured by the ulcer index, percentage of lesion area and mucus production. Histopathology and antioxidant were also evaluated after treatment to gain additional data regarding the gastroprotective effect of powder fraction of leaves of *A. africana* in the rats’ stomachs.

3.1. Gastroprotective effect of powder fraction of leaves of *A. africana* on the gastric mucosa

Photographs of the stomachs from each group are presented in Figure 1. As shown in Figure 1A, a completely healthy pink gastric mucosa with normal thickness was found in the stomach of normal rats. The rats in the ulcer control group displayed critical tissue reactions with long dark red submucosal haemorrhagic stripes and mucosal thickening, indicating that alcohol-induced gastric ulcer was successful. Oral pre-treatment with famotidin or different doses of powder fraction of leaves of *A. africana* weakened the gastric lesions, including their number and length. Noteworthy, haemorrhages, thickening and congestion were hardly found in the high dose of *A. africana* group, and the mucosa colour was pink, like normal.

The ulcer control group presented severe mucosal injury with an average ulcer index of 7.46 ± 0.27. A significant decrease in ulcer index was observed in the Famotidin-treated group with an average index of 4.72 ± 0.47. For the powder fraction of leaves of *A. africana*-treated groups, the ulcer index was significantly attenuated in a dose-dependent manner. The 400 mg/kg of the powder fraction of *A. africana* group exhibited the smallest ulcer index (2.27 ± 0.70) and the highest inhibition. This was accompanied by an increase of mucus production for all treated groups. A representative stomach of each group is shown in figure 1, the ulcer index, percentage ulcerated area and mucus production are listed in Table 1.

To quantitatively evaluate the protective effect of the fraction powder of leaves of *A. africana*, the ulcerated area percentage (%) and ulcer inhibition percentage were calculated, as shown in Table 1. A remarkable decrease in ulcerated areas was observed after the powder fraction of leaves of *A. africana* administration. The ulcer percentage of inhibition was greater than 70% at dose of 400 mg/kg. The results demonstrated that the powder fraction of leaves of *A. africana* could protect the gastric mucosa against acute injury caused by ethanol and was even better than famotidin.

![Figure 1](image)

**Figure 1** Effects of the powder fraction of leaves of *A. africana* on the macroscopic appearance of the gastric mucosa in the ethanol-induced gastric lesions in rats. (A) Normal group; (B) Control group; (C) AA (100 mg/kg) group; (D) AA (200 mg/kg) group; (E) AA (400 mg/kg) group and (F) Famotidin group (100 mg/kg)
Table 1 Effect of the powder fraction of leaves of *A. africana* on gastric lesions induced by absolute ethanol.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>N</th>
<th>Ulcer index (IU)</th>
<th>% Ulcerated area</th>
<th>% Inhibition</th>
<th>Mucus production (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>-</td>
<td>5</td>
<td>0.00</td>
<td>-</td>
<td>-</td>
<td>89.42</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>5</td>
<td>7.46 ± 0.27</td>
<td>1.11</td>
<td>-</td>
<td>116.61</td>
</tr>
<tr>
<td><em>A. africana</em></td>
<td>100</td>
<td>5</td>
<td>4.23 ± 0.20***</td>
<td>0.63</td>
<td>43.25</td>
<td>122.8 ± 5.01</td>
</tr>
<tr>
<td><em>A. africana</em></td>
<td>200</td>
<td>5</td>
<td>2.89 ± 0.24***</td>
<td>0.43</td>
<td>61.22</td>
<td>134.2 ± 5.95*</td>
</tr>
<tr>
<td><em>A. africana</em></td>
<td>400</td>
<td>5</td>
<td>2.27 ± 0.70***</td>
<td>0.34</td>
<td>70.96</td>
<td>161.2 ± 7.97**</td>
</tr>
<tr>
<td>Famotidine</td>
<td>100</td>
<td>5</td>
<td>4.72 ± 0.47***</td>
<td>0.70</td>
<td>36.73</td>
<td>100 ± 3.98</td>
</tr>
</tbody>
</table>

Values are represented as the mean ± SEM (n=5). *p < 0.05; **p < 0.01 and ***p < 0.001 indicate significant difference compared to control.

3.2. Histopathological Analysis

Histopathological observations of normal control gastric tissue specimen exhibited a complete structure of gastric mucosa, tidily arranged epitheliums and gastric glands with shape rules (Figure 2). In contrast to control group, ethanol administration had manifested extensive gastric lesions accompanied with intense desquamation of epithelial, necrosis, and hemorrhages such as inflammatory cells infiltration and submucosal edema. However, the powder fraction of leaves of *A. africana* pretreatment minimized the pathologic changes and effectively protected the stomach lining via reducing inflammatory cell infiltration and submucosal edema in a dose-dependent manner. Administering of Famotidine at 100 mg/kg significantly ameliorated the gastric histopathological changes by recovery in mucosal epithelium and regeneration glandular structure despite the present of edema.

![Figure 2](image)

The influence of the powder fraction of *A. africana* on ethanol-induced oxidative stress was studied by testing SOD, CAT, GSH and MDA in different groups. In the present study, the ulcerated rats after administration of ethanol significantly reduced gastric GSH and CAT.
A

\[ \alpha p < 0.01 \] ; et \[ \alpha\alpha\alpha p < 0.001 \] : Statistically significant compared to Control; Control (spontaneous healing)

B

\[ p < 0.05 \] : Statistically significant compared to Control; Control (spontaneous healing)

C

Control (spontaneous healing)
Figure 3 Effect of the powder fraction of leaves of *A. africana* on the level of GSH (A), CAT (B), SOD (C) and MDA (D) in the gastric tissue of different treated groups. α: comparing with the ulcer control group

4. Discussion

The present study aimed to evaluate the anti-ulcer activity of the powder fraction of leaves of *A. africana* using an ethanol-induced experimental gastric ulcer model and to investigate its underlying mechanisms of the action associated with its anti-ulcer activity. The experimental model of ethanol-induced gastric ulcer represents several features of human ulcerative condition; and thus, is effective for evaluating the anti-ulcer potential of drugs along with their possible implicated mechanisms [22, 23].

Ethanol induces gastric injury through different mechanisms; including acute hemorrhagic gastric erosions, and excessive ingestion can result in gastritis characterized by mucosal edema, sub-epithelial hemorrhages, cellular exfoliation, and inflammatory cell infiltration [24, 25]. Alcohol causes indirect damaging effects via the recruitment of leukocytes which drives inflammatory responses, oxidative stress, and apoptosis [26-28].

In the current study, ethanol-induced ulcerations were observed macroscopically as severe haemorrhagic lesions in stomach specimens of rats that were administered absolute ethanol (Figure 1 B). This can be explained as ethanol causes blood stasis and disrupt gastric microvessels; which inflict hemorrhage and necrotic gastric damage [29], all are the hallmarks of an acute inflammatory reaction.

However, from both macroscopic and microscopic observations, the powder fraction of leaves of *A. africana* pretreatment effectively alleviated the ethanol-induced gastric ulcers in rats. All treatment groups exhibited significant dose-dependent reduction of gastric lesion area (Figure 1) in gastric mucosa as well as increment of gastric wall mucus levels (Table 1) compared with ethanol group, which was also supported by the pathological changes such as decrement of desquamation of epithelial, submucosal edema and inflammatory cell infiltration (Figure 2).

The mucus layer lining the entire gastric mucosa acts as a physical barrier against the aggressive effect of gastric juice, aspirin, or cold restraint stress. Further, can act as an antioxidant, and thus can reduce mucosal damage mediated by oxygen free radicals. Thus, weakening of the mucus barrier is directly responsible for gastric damage and absolute ethanol is well known to disrupt gastric mucus layer [30, 31]. In line with this view, the powder fraction of leaves of *A. africana* shows preserved gastric mucus content comparable to the ulcer control. Thus, it could be said that the powder fraction of leaves of *A. africana* protects gastric mucosa through the preservation of gastric mucus content.

Oxidative stress has been well linked to the pathological process of gastric ulcer [32]. Ethanol increases superoxide anion and hydroxyl radical production and lipid peroxidation in the gastric mucosa [30]. It is well known that oxygen-derived free radicals can react with lipids to form lipid peroxides, for the major components of cytomembrane are lipids, which do extensive damage. If the processes were not neutralized by sufficient antioxidant molecules, their peroxidation...
can lead to cell death and/or apoptosis [1]. Our findings were in line with those of previous studies indicating that absolute ethanol induces gastric ulcers by increasing ROS levels. The generation of high levels of ROS in gastric tissue plays a vital role in the formation of lipid peroxides, such as MDA, and is accompanied by antioxidative enzyme activity impairment in cells [33, 34]. This enzyme complex is constituted of antioxidants such as GSH and antioxidant enzymes such as SOD and CAT. Depletion in cellular GSH content as well as weakened of SOD and CAT activities could debilitate recovery after short period of ethanol induced gastric oxidative injury [35]. As shown in the results of current experiment (Figure 3), ethanol exposure causes significant diminutions in GSH content and CAT activities. Conversely, the powder fraction of leaves of *A. africana* pretreatment displayed significant rises of GSH levels and a reduction of the MDA level, specifying its antioxidant potential and further confirming that this powder fraction has gastroprotective properties against the development of ethanol-induced ulcers.

5. Conclusion

In conclusion, our present work revealed that the powder fraction of leaves of *A. africana* exerted appreciable gastroprotective effect against ethanol-induced gastric lesions in rats. This gastroprotective effect could be associated with the preservation of gastric mucus layer, the enhancement of antioxidant defense maintenance of GSH levels and reduction of lipid peroxidation and reorganized glandular.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors have not declared any conflict of interest. The authors alone are responsible for the content and writing of the paper.

Statement of ethical approval

The experimental protocols were approved by the University’s Animal Care Ethics Committee.

Author Contributions

- Research project: A. Conception, B. Organization, C. Execution.
- Manuscript: A. Writing of the first draft, B. Review and Criticism.
- Ndji: 1A, 1B, 1C, 2A, 2B; Essama: 1A, 1B, 1C; Mache: 1C; Zelock: 1C; Hamadjida: 1B, 2B; Mingoas: 2B; Njintang: 1C, 2B.

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