EVALUATION OF THE ANTIULCER PROPERTIES OF AQUEOUS AND METHANOL EXTRACTS OF VITEX DONIANA ON INDOMETHACIN INDUCED GASTRIC ULCERS IN ALBINO RATS


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Abstract

The present study was carried out to investigate the antiulcer-activity of crude aqueous and methanol extract of Vitex doniana leaves in Indomethacin induced ulcer model in experimental rats. Seven groups (A – G) of five animals were used for this study. Group A served as the baseline control, gastric ulcer was induced in all the other groups by administering a single dose of 60 mg/kg body weight of indomethacin with or without prior 3-day treatment with standard drug (cimetidine) or plant extract (low dose and high dose aqueous and methanol extract). The animals were sacrificed, stomachs excised and ulcers scored. Histological analysis was also performed. It was observed that V. doniana extracts protected the stomach of the animals from indomethacin induced ulcerations to various extents with the high dose (400 mg/kg body weight) methanol and aqueous extract having about 70% and 59% ulcer protective index respectively. Histological findings also correlated with calculated indices with treated animals having significantly less ulcer lesions when compared to untreated ones. In conclusion, extracts of V. doniana possess antiulcer properties against Indomethacin induced gastric ulcer in rats.

Keywords: Antiulcer, Vitex doniana, antioxidants, flavonoids.
Introduction

Non-steroidal anti-inflammatory drug (NSAID) are important for management of pain as a result of arthritis and musculoskeletal disorders. These class of drugs are however known to induce gastric mucosal damage and has been linked development of peptic ulcer disease in about 25% of chronic users. Development of gastric ulcers among NSAID users has been linked to the inhibition of cyclooxygenase -1 (COX – 1) by the drug. This inhibition leads to marked decrease in prostaglandin (a known gastro-protective factor) production (1, 2).

Plants are a major source of new medicines and are widely used by man to treat various ailments (3). Vitex doniana is a widely distributed tree in southern and eastern Nigeria. Where the plant is found, many of the locals use the plants for various purposes including for treatment of various gastrointestinal and other diseases (4). Previous works by several authors have shown that V. doniana possess desirable antioxidant (5) and antimicrobial (6) properties which are both important in the strategies currently employed for the treatment of peptic ulcer (7, 8). These reported properties were attributed to the presence of various phytochemicals present in the plant.

Even though many NSAID alternatives exist, these class of drugs are still in use by a large number of people hence the need to find new ways of mitigating their potential for causing gastrointestinal damage. Indeed, the aqueous and methanol extracts of V. doniana have been shown to possess anti-ulcer properties against ethanol induced gastric ulcers in experimental animals (9). However to the best of our knowledge, the ability of the extracts to protect against NSAID induced gastric ulcers is yet to be reported.

Materials and Methods

Experimental Animals

Apparently healthy, disease free albino rats (n = 35, weighing 146 – 221 g) were obtained from and housed in metallic cages at the Animal House of the College of Medicine, University of Nigeria, Enugu Campus (UNEC). The animals were left to acclimatize to the laboratory conditions of ambient temperature (28 - 30°C) and a 12 hour light-dark cycle. They were weighed and distributed into 7 groups of 5 animals according to weights. They were fed on standard commercial rat feed (Guinea Feed®, Enugu, Nigeria) and clean water ad libitum. Animal handling, care and experimentation was done under close supervision of animal care staff of the College of Medicine, UNEC to ensure adherence to the guidelines for the use of animals in research (10).

Plant Collection, taxonomy and extraction

V. doniana leaves were collected within Enugu, Enugu state Nigeria in the month of August, 2016. Plant identity was confirmed by a specialist at the Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences, University of Nigeria Nsukka (voucher number PxUNN/098). The leaves were thoroughly washed and dried under shade after which they were ground into fine powder.

For the aqueous extract (AEVD), 100 g of the powder were soaked in 400 ml of clean water for 48 hours to extract in a refrigerator. The mixture was intermittently agitated during this period to improve extraction. After 48 hours, the mixture was sieved using a muslin cloth and Whatmann filter paper and the obtained filtrate was stored in the refrigerator at 2–8°C until required for use.

For the methanol extract (MEVD), another 100g of the powder were dissolved in 500 ml of methanol for 72 hours. The mixture was left in a refrigerator and allowed to extract with intermittent agitation. After the extraction period, the mixture was filtered using Whatman filter paper and the filtrate allowed to evaporate to dryness. The remaining concentrate was dissolved in 3% Tween 80 and stored in the refrigerator till needed.

Induction of gastric ulcers using Indomethacin

A single dose of indomethacin at 60 mg/kg body weight was used to induce the ulcers. All animals except those in Group A (baseline control) received a single oral dose of indomethacin after 24 hours of food deprivation with or without prior treatment with either standard drug or extract. Group B (the negative control) did not receive any treatment prior to ulcer induction; Group C (positive control) received 100 mg/kg body weight (bw) of cimetidine intramuscularly for 3 days prior to the commencement of the experiment and 40 minutes before the ulcer induction. Groups D and E received 200 mg/kg bw and 400 mg/kg bw of MEVD respectively while Groups F and G received 200 mg/kg bw and 400 mg/kg bw of AEVD respectively for 3 days prior to commencement of experiment and 40 minutes before ulcer induction.

Calculation of Ulcer Index

After 4 hrs the animals were sacrificed under, their stomachs were removed, rinsed in saline and opened along the greater curvature. The ulcers were counted with the aid of a magnifying lens. The ulcerative lesion index was calculated as follows: ulcerative lesion < 1 mm = 1; ulcerative lesion > 1 mm < 2 mm = 2; ulcerative lesion > 2 mm = 3. The sum of the scores was divided by 10 to give the ulcer index for each rat. The effectiveness of the extract and drug was calculated using the formula:

Ulcer preventive index (UPI) (%) = Ulcer index of control - Ulcer index of treated/Ulcer index of control × 100(11, 12).

Plant Collection, taxonomy and extraction

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The excised stomach tissues were processed into formalin fixed, paraffin sections. The slides were stained using the haematoxylin and eosin technique. The stained stomach sections were examined using a Magnus™ microscope, micrographs of the sections were obtained using a Samsung NX1000 digital camera attached to the microscope.

Statistical Analysis
Data analysis was done using SPSS version 20 with results expressed as mean ± SEM. Statistical comparison between treatment and negative control groups was performed using the Dunnett’s test with significance set at P < 0.05.

Results
Ulcer index and ulcer preventive index
Gross examination of the excised stomach tissues revealed varying degrees of mucosal ulcerations (Figure 1). The calculated mean ulcer indices (UI) and ulcer preventive indices (UPI) for the test animals are summarised in Table 1 below. Group B had the highest UI of 2.3 ± 0.15 whereas all the animals pre-treated with either cimetidine or extracts had significantly lower UI in comparison. The group of animals treated with 400 mg/kg bw of MEVD had the least UI (0.67 ± 0.12) and highest UPI (70%) showing that MEVD at that dose afforded the highest protection against gastric ulcerations. Both extracts at 400 mg/kg bw afforded better gastro-protection (UPI = 70% for MEVD and 59% for AEVD) than cimetidine (UPI 54%) which is a standard anti-ulcer drug.

Tissue histopathology
Induced ulcers presented histologically as areas of epithelial erosion in the gastric mucosa of the test groups (depicted by the arrows in figure 2). Animals in Group B (negative control) appeared to be the most affected with several zones of severe erosion (Fig 2b). Sections of stomach tissue from Group D and F also showed severe erosions accompanied by purulent exudation (Fig 2d) and infiltration of mucosa by inflammatory cells (Fig 2f). Groups C (Cimetidine control), E (high dose MEVD) and G (high dose AEVD) appeared to have the least number of eroded areas in their gastric epithelia (Fig 2c, e, and g).

Discussion
This study was designed to evaluate the antiulcer property of crude aqueous and methanol extracts of Vitex doniana in indomethacin induced rodent gastric ulcer model. A single dose of indomethacin at 60 mg/kg body weight was sufficient to cause significant gastric mucosal injury.

Indomethacin and other NSAIDs are well known for their ability to cause gastric mucosal damage (13). A major mechanism of achieving this is by suppressing prostaglandin production via cyclooxygenase inhibition (14). Prostaglandins play a crucial role in maintaining gastric mucosal integrity by stimulating mucus and bicarbonate production, increasing mucosal perfusion, suppressing acidity and hastening gastric epithelial turnover (15,16). Suppression of prostaglandin increases the susceptibility of the gastric mucosa to damage by causing gastric hypermotility, disrupting gastric blood flow, stimulating reactive oxygen species, lipid peroxidation and neutrophil infiltration (17, 18).

Pre-treatment of the experimental animals with either cimetidine or extracts reduced the extent of gastric mucosal damage induced by indomethacin. The excised stomachs of the pre-treated animals had less mucosal lesions and compared to the negative control. Also, the mean ulcer indices and ulcer protective indices shows that the extracts were able to protect the stomach significantly. The protection afforded by the extracts appear to be dose dependent with the higher doses having higher percentage ulcer preventive indices.

Cimetidine, an active histamine H2 receptor antagonist, is a standard drug used in treating peptic ulcer. It helps in treatment and healing of gastric ulcers by suppressing the production of gastric acids (19). V. doniana is known to contain many phytochemicals including alkaloids, tannins, flavonoids, phenolic compounds, steroids, resins, glycosides, essential fatty acids (20) which may be responsible for the gastroprotection observed. These polyphenols are known to possess cytoprotective abilities and thus antiulcer properties. For instance, tannins, with their ability to precipitate microproteins and cause vasoconstriction have been suggested to confer gastric protection by creating an extra protective layer when applied to gastric mucous membranes making it less susceptible to damage by irritants (16). Saponins have also been reported to possess antiulcer properties (21) possibly due to its surfactant-like properties. Also, flavonoids are well known antioxidants able to confer gastric mucosal protection by their ability to clear our reactive oxygen species, stabilize mucous membranes (22) and increase gastric mucosal prostaglandin (23).

The antiulcer property of V. doniana extracts may have been exerted by one or more of the above discussed mechanisms thus providing scientific support for the folkloric use of V. doniana for treating stomach ailments. Our result also suggest the possibility of using V doniana extracts as a basis for developing alternative drugs for treating gastric ulcers. More studies are ongoing to unravel more precise mechanisms of action involved in the observed activity.

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References
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Table 1 Ulcer indices and ulcer preventive indices of the various treatment groups prior to ulcer induction by Indomethacin

<table>
<thead>
<tr>
<th>GROUP</th>
<th>TREATMENT</th>
<th>DOSES (mg/kg body weight)</th>
<th>ULCER INDEX*</th>
<th>P VALUE</th>
<th>ULCER PREVENTIVE INDEX (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>None</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>B</td>
<td>Water</td>
<td>1ml/animal</td>
<td>2.3 ± 0.15</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C</td>
<td>Cimetidine</td>
<td>100</td>
<td>1.03 ± 0.67</td>
<td>0.016</td>
<td>54</td>
</tr>
<tr>
<td>D</td>
<td>MEVD</td>
<td>200</td>
<td>1.13 ± 0.57</td>
<td>0.027</td>
<td>27</td>
</tr>
<tr>
<td>E</td>
<td>MEVD</td>
<td>400</td>
<td>0.67 ± 0.12</td>
<td>0.003</td>
<td>70</td>
</tr>
<tr>
<td>F</td>
<td>AEVD</td>
<td>200</td>
<td>1.23 ± 0.12</td>
<td>0.044</td>
<td>46</td>
</tr>
<tr>
<td>G</td>
<td>AEVD</td>
<td>400</td>
<td>0.8 ± 0.09</td>
<td>0.005</td>
<td>59</td>
</tr>
</tbody>
</table>

*results shown are presented as mean ± SEM (n = 5 rats per group). # Value significantly different from group B (negative control) at p < 0.05.

Figure 1 Macrographs showing the gross appearance of the excised stomach mucosae of rats in different treatment groups. Ulcers are indicated by black arrows. Group A is the baseline group and had no ulcers. Group B (60mg/kg indomethacin only) showing high number of haemorrhagic spot ulceration of the stomach mucosa; Group C (100mg/kg Cimetidine) shows mild streak haemorrhagic ulcerations; Group D (200mg/kg MEVD) showing moderately ulcerations; Group E (400 mg/kg MEVD) showing just one spot ulcerations; Group F (200mg/kg AEVD) and G (400 mg/kg AEVD) showing moderate ulcerations of the gastric mucosa.
Figure 2: Photomicrographs showing the ulcers of the excised stomach mucosae of rats in different treatment groups histologically. Ulcers are indicated by black arrows. Group A is the baseline group and had no ulcers. Group B (60mg/kg indomethacin only) shows severe erosion of the gastric epithelia; Group C (100mg/kg Cimetidine) shows mild gastric epithelial erosions; Group D (200mg/kg MEVD) shows an area of severe erosion filled with purulent matter; Group E (400 mg/kg MEVD) shows very mild erosion of the gastric epithelia; Group F (200mg/kg AEVD) and G (400 mg/kg AEVD) shows severe and moderate erosions of the gastric epithelia respectively. All sections are stained with Haematoxylin and Eosin (H & E). All sections are viewed at a total magnification of X100.