Comparing *Ficus vogelii* leaf extract and omeprazole as therapy and prophylaxis for aspirin-induced gastric ulcer in wistar rat.

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Abstract

**Background:** A proper attention is required to prevent the gastric mucosa from injurious effect of aspirin.

**Objective:** We aim to examine the phytochemicals in aqueous extract of *Ficus vogelii* leaf (F.V.E), and compare their actions on aspirin-induced gastric ulcer with that of Omeprazole.

**Materials and Methods:** Thirty wistar rats (about 8 wks old and weight, 120g each) were divided into 6 groups of five rats each. With the aid of oro-gastric cannula, the groups were administered a daily dose of Omeprazole and F.V.E as follows: group 1 (negative control)-2 ml of distilled water; group 2– Omeprazole 20 mg/kg body weight (bwt) for 10 days, followed by aspirin 500 mg/kg bwt for 3 days; group 3-aspirin 500 mg/kg bwt for 3 days, followed by F.V.E 600 mg/kg bwt for 10 days; group 4-aspirin 500 mg/kg bwt for 3 days, followed by Omeprazole 20 mg/kg bwt for 10 days; group 5-F.V.E 600 mg/kg bwt for 10 days, followed by aspirin 500 mg/kg bwt for 3 days; group 6 (positive control)-aspirin 500 mg/kg bwt for 3 days. The rats were sacrificed and the stomach tissues were processed, stained with H & E and PAS, and biochemically analyzed for NP-SH group concentration.

**Results:** F.V.E has high concentrations of saponins and flavonoids. Rats in group 1, 4 and 5 showed normal gastric mucosa. Group 2 and 6 showed gastric ulceration with significant increase in parietal cell count (p<0.01), and reduction in surface cell diameter and count. Group 3 showed evidence of regenerated mucosa. All groups except positive control had normal NP-SH group concentration.

**Conclusion:** The study proved that F.V.E could be used as both therapy and prophylaxis for aspirin-induced gastric ulcer in wistar rat while omeprazole adequately served as a therapy for the ulcer.

**Keywords:** Gastric mucosa, Histopathology, Non-protein sulphydryl group, Periodic acid Schiff, Prophylaxis, Steroids.

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Introduction

The prevalence and severity of aspirin related gastrointestinal injury, requires efforts being directed at the prevention of such complication, since there are enormous health benefits of aspirin administration. Previous researchers have established widely accepted mechanism of action of aspirin in relation to gastric mucosa ulceration [1-3] and anti-inflammatory effects of *Ficus vogelii* leaf extract in some experimental animals [4,5]. Despite the fact that there are available proton pump inhibitors such as Omeprazole, targeting gastric ulceration therapy, potent prophylactic drugs for aspirin induced ulceration are required. Facts from this study may introduce new targets of pharmacologic agents to address the above proposal.

Consistently, copious literature [6-8] has established the gastroprotective or anti-ulcerative effects of different plant extracts. As such, botanical compounds with anti-ulcerative effect includes Flavonoids, Saponin, Tannins, Gums and Mucilages which have been isolated from plants such as *Aloe vera* [9], *Ficus arnottiana* [10], *Magnifera indica* and *Moringa pterygosperma* [11], *Ficus exasperate* [12], *Aegles memolos* and *Ficus religiosa* [13] and *Ficus vogelii* [4]. Notably, administration of crude extract of *Ficus thonningii* for 7 days led to a significant increase in the stomach mass, and mucosal layer thickness [14].

Interestingly, *Ficus vogelii* has wild ethno medical applications, and it serves as vegetable to prepare different types of dishes, among different tribes in Nigeria, which include the Ikwo Noyo clan people of Ebonyi State, and Obudu people of cross river state [4]. The leaf extract boost the haemoglobin level in children, and increases their body weight [4,5]. However, literature reveals lack of uniformity in the chemical and physical qualities of an herb [15-17].

Hence, the aim of this study was to ascertain the different concentrations of the basic phytochemicals in aqueous extract of *Ficus vogelii* leaf found at Ebony State, Nigeria. Furthermore, compare its prophylactic and therapeutic actions on aspirin induced gastric ulcer in Wistar rat with that of Omeprazole.
Material and Methods

Plant preparation and extraction

Fresh leaves of *Ficus vogelii* were harvested from three different resident compounds in Ndufu Alike, Ikwo Local Government area of Ebonyi State. The plant leaves were authenticated by a botanist and shade dried for three weeks, and blended into a fine powder using a Q-link electric blender (Model QBL-18L40), and stored in air-tight containers. Five hundred grams (500 g) was soaked in 1200 ml of water (powder/solvent), and agitated using an electric blender (to enhance proper mixing of the solvent with the powder), and then poured into air-tight plastic container. The container was kept in the refrigerator at 40°C for 8hours, followed by filtration with what man’s No 1 filter paper (24 cm). The filtrate was concentrated using rotary evaporator (Model QBL-18L40), and stored in air-tight containers. Five hundred grams (500 g) was soaked in 1200 ml of water (powder/solvent), and agitated using an electric blender (to enhance proper mixing of the solvent with the powder), and then poured into air-tight plastic container. The container was kept in the refrigerator at 40°C for 8hours, followed by filtration with what man’s No 1 filter paper (24 cm). The filtrate was concentrated using rotary evaporator (Model QBL-18L40), and stored in air-tight containers. Five hundred grams (500 g) was soaked in 1200 ml of water (powder/solvent), and agitated using an electric blender (to enhance proper mixing of the solvent with the powder), and then poured into air-tight plastic container. The container was kept in the refrigerator at 40°C for 8hours, followed by filtration with what man’s No 1 filter paper (24 cm). The filtrate was concentrated using rotary evaporator (Model QBL-18L40), and stored in air-tight containers.

Qualitative and quantitative phytochemical analyses of the extract

Test for tannins: A mixture of 0.5 g of the extract and 20 ml of distilled water was boiled in a test tube and filtered using a conical flask and filter paper. Formation of blue black coloration when 0.1% FeCl₃ solution was added to the filtrate indicated the presence of tannins. The quantity of tannins in the extract was determined following the methods of Van-Burden and Robinson [18].

Test for saponins: A mixture of 2 g of extract and 20 ml of distilled water was boiled in a test tube and filtered. 10 ml of the filtrate and 5 ml of distilled water was mixed in a test tube and shaken vigorously to obtain a stable persistent froth. Formation of emulsion when 3 drops of olive oil was mixed with the froth indicated the presence of saponins. The quantity of saponin in the extract was determined following the methods of Obdoni and Ochuko [19].

Test for flavonoids: Three drops of 1% ammonia solution was added to a mixture of 10 ml distilled water and 1 g of extract in a test tube. A yellow coloration indicated the presence of flavonoid compounds. The quantitative analysis was based on the formation of the flavonoids-aluminium complex which has a maximum absorption at 415 nm, as adopted by Laloo D and Sahu AN [20].

Test for steroids: The appearance of a violet coloration that changed to blue in a mixture of 2 ml of acetic acid, 2 ml methanol, 0.5 g of the extract and 2 ml H₂SO₄ confirmed the presence of steroids. The quantity of steroid in the extract was determined in a mixture containing 1 ml of the extract, sulfuric acid (4N, 2 ml) and iron (III) chloride (0.5 w/v, 2 ml). Potassium hexacyanoferrate (III) solution (0.5% w/v, 0.5 ml) was added to the mixture, and it was heated in a water bath at 70°C for 30 minutes with occasional shaking and diluted to the mark of 10 ml in a volumetric flask with distilled water. The absorbance was measured at 780nm against the reagent blank.

Steroid concentration = (Average absorbance × volume of extract × 100)/ (weight of sample × 1000)

Alkaloids: A mixture of 0.2 g of extract and 2% H₂SO₄ was warmed for two minutes, filtered and then three drops of Dragendorff’s reagent was added. Formation of orange-red precipitate indicated the presence of alkaloids. The quantity of alkaloid in the extract was determined using the method adopted by Harborne [21].

Experimental animals

Thirty (30) Wister rats were obtained from the department of animal husbandry Ebonyi State University. They were kept during acclimatization and treatment periods at the animal house of the department of biological sciences, Alex Ekwueme Federal University Ndufu Alike Ikwo. They were maintained under standard laboratory conditions (22-28°C, 60-70% relative humidity, 12:12 h light/dark cycle) with Standard Top Food grower mash and water ad-libitum. The rats were fasted for 24hrs before commencement of aspirin (500 mg/kg body weight) administration.

Experimental procedure

Thirty wistar rats (about 8 wks old and weight, 120 g each) were divided into 6 groups of five rats each. With the aid of an oro-gastric cannula, Omeprazole or the extract mixed with 2 ml distill water was administered to the groups as follows: group 1 (negative control) received 2 ml of distil water daily; group 2 received 20 mg/kg body weight (bwt) of Omeprazole daily for 3 days; group 3 received 500 mg/kg bwt of aspirin daily for 3days, followed by 600 mg/kg bwt of F.V.E daily for 10 days; group 4 received 500 mg/kg bwt of aspirin daily for 3 days, followed by 20 mg/kg bwt of Omeprazole daily for 10 days; group 5 received 600 mg/kg bwt of F.V.E daily for 10 days, followed by 500 mg/kg bwt of aspirin daily for 3 days; group 6 (positive control) received 500 mg/kg bwt of aspirin daily for 3 days. The rats were sacrificed by cervical dislocation and their stomachs were excised and carefully opened along the line of the greater curvature to expose the mucosa.

The stomach was rinsed and fixed in 10% neutral buffered formalin (pH 7.0), dehydrated in ethyl alcohol, then cleared in xylol and embedded in paraffin; 4-6 microns thickness sections obtained and stained with heamatoxylin, eosin and periodic acid Schiff for microscopic examination. The count of cells lining fundic glands and measurement of their diameters were done using CAS 200 image analyzer computer system. The diameters of surface cell and parietal cells were measured; 10 cells per section were observed at X 40 objective by two independent observers. The cell diameter was measured as the average of two diagonal diameters. The count of the cells was done at 40x objective in 10x none overlapping fields for each section.

Biochemical analysis

Determination of NP-SH: When the rats were sacrificed, the glandular stomachs were opened and rinsed in ice-cold saline. The tissues were homogenized in TCA solution. The homogenates were centrifuged in ice-cold, and separated into supernatants and pellets. A mixture of the supernatants and 5, 5’-dithiobis (2-nitrobenzoic acid), in phosphate buffer saline containing EDTA (pH 7.4) was incubated. Absorption at 412 nm was read 5 min after it was incubated using Model 6305 (Jenway, USA) spectrometer with a range of 198-1000nm.

Statistical analysis

Analysis of data was done using computer statistical package for social sciences version 20.0. The level of homogeneity among the groups was tested using analysis of variance (Snedecor and Cochran). Where heterogeneity occurred, the groups were separated using Duncan multiple range test (DMRT). A value of p<0.05 or p<0.01 indicated a significant difference between groups.

Results

The results are discussed in the following figures and tables in detail (Figures 1-8 and Tables 1-3).

Phytochemical analysis

Table 1 shows concentrations of the basic phytochemicals in aqueous extract of Ficus vogelii leaf found at Ebonyi State, Nigeria.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Qualitative</th>
<th>Quantity (mg/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saponin</td>
<td>+++</td>
<td>3.63</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>0.82</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>0.89</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+++</td>
<td>3.32</td>
</tr>
<tr>
<td>Steroids</td>
<td>++</td>
<td>2.3</td>
</tr>
<tr>
<td><strong>(Minute), ++(Less abundant), +++(Much abundant)</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Biochemical results

Table 2 shows effects of aqueous extract of Ficus vogelii leaf and Omeprazole on non-protein sulfhydryl groups (NP-SH) of Wistar rats treated with Aspirin.

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>NP-SH (nmol/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1682.57 ± 27.94</td>
</tr>
<tr>
<td>2</td>
<td>1473.57 ± 102.16</td>
</tr>
<tr>
<td>3</td>
<td>989.46 ± 12.81</td>
</tr>
<tr>
<td>4</td>
<td>1466.71 ± 49.35</td>
</tr>
<tr>
<td>5</td>
<td>1385.76 ± 63.99</td>
</tr>
<tr>
<td>6</td>
<td>896.42 ± 6.66  **</td>
</tr>
</tbody>
</table>

**Represents a significant reduction (p<0.01) when compared with negative control (Group 1). * Represent a significant increase (p<0.05) and (p<0.01) respectively, when compared with the positive control (Group 6). Values are means ± SD. n=5 in each group.

Discussion

Aspirin is used for both therapeutics (to reduce pain, inflammation and fever) and prophylactics (to prevent thrombotic events). Although prophylactic doses of aspirin are generally lower than therapeutic doses, epidemiologic studies suggest that such doses may still be associated with gastrointestinal damage [22]. The dose chosen in this study was similar to the dose chosen by other researchers [23,24], which induced gastric ulceration while used in treatment of osteoarthritis. The pharmacological dynamics and kinetics of Omeprazole has been extensively discussed in the literature. On the other hand, the dosage of FVE used in this study was in the range of values adopted by [4]. That had anti-inflammatory effects in rats. The upper part of the fundic region of the stomach was chosen because it is the most common region for gastric ulcers [25].

Aspirin administration was accompanied by different forms of gastric mucosal lesions together with collagen fibre deposition in the lamina propria of rats in group 2 & 6 (Figures 2 and 6). The findings in group 2 revealed that pre-treatment with Omeprazole could not protect the gastric mucosa from damage induced by aspirin. However, its parietal cell count and that of other test groups, with exception of positive control group could not show a significant difference with the negative control group. In addition, it revealed a relatively larger surface cell diameter and parietal cell diameter when compared with the positive control group (Table 2). This remarkable structural change of the parietal cells and maintenance of its count within the normal range could be as a result of the fact that Omeprazole has short half-life (0.5-1 hour), slow onset and long-acting pharmacodynamic effect of acid secretion reduction at baseline over 3-5 days.

In support of this result, [26,27] stated that erosion may develop as an ischemic infarct following a therapeutic dose of aspirin. Since, the first structural change in rats is the damage of basement membrane of the endothelial cells of the capillary
and post capillary venule. It leads to breakdown of small blood vessels before any other cytolysis. Aspirin administration led to increased fibrin deposition in the gastric mucosa [28]. Authors [29-31] detected variable lesions in the gastric mucosal cells following treatment with therapeutic doses of aspirin.

Table 3. Effects of aqueous extract of Ficus vogelii leaf or Omeprazole on gastric surface and parietal cells of wistar rats treated with Aspirin.

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Surface cell count</th>
<th>Surface cell diameter</th>
<th>Parietal cell count</th>
<th>Parietal cell diameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>22.26 ± 2.47</td>
<td>13.52 ± 0.51</td>
<td>11.81 ± 0.29</td>
<td>20.85 ± 0.81</td>
</tr>
<tr>
<td>2</td>
<td>17.52 ± 1.22</td>
<td>11.05 ± 0.83</td>
<td>12.37 ± 0.16</td>
<td>19.04 ± 0.51</td>
</tr>
<tr>
<td>3</td>
<td>14.82 ± 2.67</td>
<td>10.44 ± 0.45</td>
<td>12.52 ± 1.47</td>
<td>20.41 ± 0.71</td>
</tr>
<tr>
<td>4</td>
<td>19.63 ± 0.65</td>
<td>12.46 ± 0.45</td>
<td>14.20 ± 1.03</td>
<td>18.58 ± 0.36</td>
</tr>
<tr>
<td>5</td>
<td>18.15 ± 1.76</td>
<td>12.94 ± 0.67</td>
<td>13.03 ± 0.98</td>
<td>19.10 ± 0.62</td>
</tr>
<tr>
<td>6</td>
<td>12.30 ± 1.51</td>
<td>8.67 ± 0.94</td>
<td>18.39 ± 0.82</td>
<td>14.59 ± 0.54</td>
</tr>
</tbody>
</table>

a,b Represent significant increase or reduction (p<0.05) and (p<0.01) respectively when compared with the negative control (Group 1). a,b Represent significant increase or reduction (p<0.05) and (p<0.01) respectively when compared with the positive control (Group 6). Values are means ± SD. n=5 in each group.

Photomicrographs showing the effects of aqueous extract of Ficus vogelii leaf or Omeprazole on gastric mucosa of wistar rats treated with Aspirin as shown in Figures 1-8.

Figure 1. Photomicrograph of a cross-section of the stomach of a rat in group 1 (negative control), showing strong PAS positive reaction with normal Surface columnar Epithelium (SCE), parietal cells and chief cells (PCells and Ccells) and Gastric pits (GP). Stain: PAS; Magnification: 200x.

Figure 2. Photomicrograph of a cross-section of the stomach of a rat in group 2, showing degeneration of surface columnar epithelium (SCE), increase in number of parietal and chief cells (PCells and Ccells) and increased fibrin deposition in lamina propria (LP). Stain: H&E; Magnification: 200x.

Figure 3. Photomicrograph of a cross-section of the stomach of a rat in group 3, showing normal or lesion regenerated area on surface columnar epithelium (SCE) and Gastric pits (GP), normal count of parietal and chief cells (PCells and Ccells). Stain: H&E; Magnification: 200x.

Figure 4. Photomicrograph of a cross-section of the stomach of a rat in group 4, showing normal surface columnar epithelium (SCE), parietal and chief cells (PCells and Ccells), and Gastric pits (GP). Stain: H&E; Magnification: 200x.
Comparing *Ficus vogelii* leaf extract and omeprazole as therapy and prophylaxis for aspirin-induced gastric ulcer in wistar rat.

**Figure 5.** Photomicrograph of a cross-section of the stomach of a rat in group 4, showing strong PAS positive reaction with normal surface columnar epithelium (SCE), normal count of parietal cells and chief cells (PCells and CCells) and gastric pits (GP). Stain: PAS; Magnification: 200x.

**Figure 6.** Photomicrograph of a cross-section of the stomach of a rat in group 5, showing normal surface columnar epithelium (SCE), parietal and chief cells (PCells and CCells) and gastric pits (GP). Stain: H&E; Magnification: 200x.

**Figure 7.** Photomicrograph of a cross-section of the stomach of a rat in group 5, showing strong PAS positive reaction with normal surface columnar epithelium (SCE), parietal cells and chief cells (PCells and CCells) and gastric pits (GP). Stain: PAS; Magnification: 200x.

**Figure 8.** Photomicrograph of a cross-section of the stomach of a rat in group 6, showing (black arrows) gastric ulceration, degenerated surface columnar epithelium, increase in the number of parietal and chief cells, fibrin deposition and bleeding in lamina propria and dilated gastric pits. Stain: PAS; Magnification: 200x.

The result (Table 2) showed that there was no significant change in the concentration of non-protein sulphhydryl group among the treatment groups, with the exception of group 6 (positive control) which showed a significant reduction in its concentration. The endogenous non-protein sulfhydryl groups present in the mucus and some enzymes of the antioxidant system participate in the production of gastric mucus and bind to the free radicals formed during inflammation [32,33] revealed that exposure of gastric mucosa to toxic concentrations of chemicals caused a rapid loss of total protein sulphurhydryl group. Therefore, FVE could have protected the gastric mucosa of Wister rats treated with aspirin by maintenance of normal concentration of non-protein sulphhydryl group.

Interestingly, light microscopic findings on the slides stained with PAS and H & E showed normal structure of surface epithelial cells, lamina propria, parietal and chief cells in negative control group (Figure 1), group 5 (Figures 6 and 7) and group 4 (Figures 4 and 5) with concentrated pink coloration of the mucus on the photomicrographs. Moreover, the therapeutic effect of concomitant administration of aspirin and FVE to rats in group 3 (Figure 3) revealed a normal or lesion regenerated area on surface columnar epithelium. PAS staining technique was adopted since it is mainly used to identify mucus cells, connective tissue and basal lamina.

Noteworthy, this result was in agreement with [24,34]. Aspirin-FVE induces less gastric mucosal damage due to the increase in expression and activity of hemeoxygenase-1 (HO-1) [35]. HO-1 plays an important role in gastro-protection against NSAID by making cells more resistant to apoptotic death [36,37]. Probably, the above actions of FVE were potentiated by its moderate concentration of steroid, which ultimately could contribute to steroidal anti-inflammatory response [38].

The result of phytochemical analysis showed a relatively, high concentration of saponins and flavonoids in FVE (Table 1). Plant-originated flavonoids are highly gastro-protective. Probably, due to enhancement of the expression of nitric oxide synthase and release of calcitonin gene related peptide by...
sensory afferent nerves that increases gastric microcirculation. Equally noted that some flavonoids were able to simultaneously inhibit the production of inflammatory prostaglandin E2 and pro-inflammatory cytokines [39,40]. Similarly, tomato saponin, alpha-tomatine could potentiate apoptosis and eliminate cells that are abnormal and potentially dangerous [40]. Most likely, FVE saponins might have protected the gastric mucosa of Wister rats in group 3 (Figure 3) and 5 (Figures 6 and 7). Through destruction of proliferated parietal and chief cells associated with aspirin administration, with consequent reduction in acid secretion and pepsinogen. This suggestion was supported by the significant increase in number of parietal cells of Wister rats in group 6 when compared with that of negative control group. Thus, the mechanisms of actions of FVE flavonoids and saponins require lucid explanations.

**Conclusions**

- *Ficus vogelii* leaf found at Ebonyi state, Nigeria has abundant flavonoids and saponins, and their mechanisms of actions require lucid explanations.
- FVE could have protected the gastric mucosa of Wister rats treated with aspirin by maintenance of normal concentration of non-protein sulpho-hydryl group that contributes to mucus production and could bind to free radicals formed during inflammation.
- The study proved that F.V.E could be used as both therapy and prophylaxis for aspirin-induced gastric ulcer in Wistar rat while Omeprazole adequately served as a therapy for the ulcer.

**Recommendation**

Concomitant administration of aspirin and FVE to prevent and manage aspirin induced gastric ulcer in Wister rat should be subject to randomized clinical trials to ascertain the efficacy.

**References**

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