Assessment of gastroprotective effect of crude tannin from *Schwenkia americana* Linn. on mitochondrial functions in ulcerogenic rats

George Edaghogho Eriyamremu, Ebehiremen Bridget Iorliam

Biochemistry Department, University of Benin, P.M.B 1154 Benin City, Edo State, Nigeria

**ARTICLE INFO**

**Article history:**
Received 8 Nov 2016
Received in revised form 28 Nov, 2nd revised form 29 Dec 2016, 3rd revised form 26 Jan, 4th revised form 31 Mar 2017
Accepted 20 Apr 2017
Available online 14 May 2017

**Keywords:**
Tannin
Gastric ulcer
Mitochondrial
*Schwenkia americana* Linn.

**ABSTRACT**

**Objective:** To investigate the gastroprotective effect of tannin from *Schwenkia americana* Linn. on mitochondrial functions in ulcerogenic rats.

**Methods:** A total number of 36 male Wistar rats weighing 160–220 g were used for the study. The rats were divided into six groups with six rats each group. Groups I and II were orally administered with distilled water, Groups III, IV and V with 50 mg/kg, 100 mg/kg and 200 mg/kg of extracted tannin whilst Group VI administered with omeprazole, respectively for 7 days. All animals were fasted for 24 h before single administration of 800 mg/kg body weight of aspirin, except for Group I (normal control). Uclegogenic activity and mitochondrial functional parameters were assessed.

**Results:** Administration of aspirin significantly (*P* < 0.05) increased free and total acidity, quantity of gastric juice, protein, pepsin activity and decreased tricarboxylic acid cycle enzymes, ATPases, fucose, sialic, hexosamine, hexose and mucin level in ulcerated rats (Group II). There was also dissipation of mitochondrial membrane potential in ulcerated rats, but prior pretreatment of extracted tannin and omeprazole prevented these biochemical effects.

**Conclusions:** Tannin from *Schwenkia americana* prevented impaired stomach mitochondrial functions in aspirin induced gastric damage and may also act as gastroprotective of the gastric mucosal.

1. **Introduction**

Non-steroidal anti-inflammatory drugs (NSAIDs) are easily available for the treatment of cardiovascular disease, arthritis and rheumatism with a less financial burden. Aspirin used for the treatment of these diseases is associated with several side effects such as inhibition of prostaglandin synthesis, alterations in mucosal permeability and gastric damage[1]. Currently, gastric ulcer therapy is facing major negative aspect because most of the drugs used for the treatment of gastrointestinal diseases are not very effective and have serious side effects[2]. Therefore, there is a need for constant development of herbal medicines for the treatment of gastrointestinal diseases[3].

Aspirin is one of the most frequently used drugs in Nigeria and the rest of the world. The elderly population are naturally weighed down with diseases like cardiovascular events, rheumatism and depression. Unfortunately, some of these drugs such as aspirin used for the treatment of these diseases decrease ATP synthesis, dissipate the mitochondrial transmembrane potential and uncouple oxidative phosphorylation[4]. Uncoupling of mitochondrial oxidative phosphorylation is the first stage in NSAIDs induced gastric ulcer[5]. Therefore, there is a need to search for alternative drugs and isolated compounds for the treatment of ulcers with less side effect[6]. In recent times, phytochemicals from plant extracts are used for developing new drugs for the treatment of gastric ulcers[7]. *Schwenkia americana* Linn. (**S. americana**) is a perennial plant, slender erect herb, woody at the base, which grows up to 1 m tall. This plant is found in uncultivated places and available in several parts of the world such as tropical America, Africa and Nigeria[8].

The aqueous and ethanol extracts of *S. americana* have been used for the treatment of gastric ulcer in rats[9]. Antiulcer activity of tannins and flavonoid is due to their antioxidant properties[10], thus prompted the extraction of tannin for the current study. The study is therefore undertaken to ascertain the effect of tannin from *S. americana* on aspirin-induced mucosal injury with special reference to the mitochondria.

2. **Materials and methods**

2.1. **Plant material and chemicals**

*S. americana* plant was obtained from farm land in Benue State, Nigeria. The plant was authenticated at Herbarium Unit University of Benin, Edo State, Nigeria. Drugs, chemicals and reagent used for this study were all of analytical grade.
2.2. Extraction and confirmatory test of tannin

Tannin was extracted according to the method of Haslam[11]. The procedure using aqueous acetone for the removal of tannins was used as indicated in Figure 1. Ferric chloride test was carried out according to the method of Doss et al.[12] to confirm the presence of tannin. The polyphenolic contents in the extracted tannin were determined using the Stiasny reaction and percentage of tannin in the crude tannin was estimated as described in the next section.

![Schematic chart for fractionation of tannin from S. americana](image)

2.3. Determination of polyphenolic and tannin content

One gram of extracted tannin was dissolved in 20 mL of distilled water, in addition; 5 mL of hydrochloric acid and 10 mL of formaldehyde (37%) were added and kept under reflux for 30 min and later filtered and washed with distilled water. The residue was placed in a drying oven at a temperature of 105 °C until mass stabilization. The percentage of polyphenol contained in the extract (Stiasny Index – SI) was determined by calculating the ratio between tannin mass and the mass of total extract, and the result converted to a percentage[13]. To determine the percentage of tannin in crude tannin extract, Stiasny index was multiplied by the total amount of extractives and the result converted to percentage.

2.4. Acute toxicity (LD₅₀) study

The toxicity of S. americana was performed in the earlier study by Eriyamremu and Iorliam[9], using the method described by Aliu and Nwude[14]. The result of acute oral toxicity (LD₅₀) of ethanolic and aqueous extracts from S. americana was found to be tolerated by rats, with no adverse effect even with administration at 3 200 mg/kg body weight.

2.5. Experimental design and animal grouping

Thirty six rats weighing between 160–220 g were used for the study. The experimental rats were obtained from University of Nigeria, Enugu, Nigeria. The animals were allowed to acclimatize for one week after their arrival to the animal house before dividing them into various groups. All experimental procedures involving animals were conducted in accordance to the standard protocols established by National Institute of Health Guide for the care and Use of Laboratory Animals and approved by Ethic Committee of the Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Groups I and II were administered with distilled water, Groups III, IV and V with 50 mg/kg, 100 mg/kg and 200 mg/kg of extracted tannin while Group VI administered with omeprazole, respectively for 7 days. All animals were fasted for 24 h before single administration of 800 mg/kg body weight of aspirin[15], except for Group I which is the normal control. After 8 h the rats were sacrificed by cervical dislocation under anaesthesia, the stomach was safely excised and the gastric contents were obtained. The contents were spun in a centrifuged at a revolution of 1 000 r/min for a period of 10 min; the centrifuged samples were decanted and used for biochemical assay. The stomach was rinsed with normal saline, whilst the ulcer scored with dissecting microscope according to the method of Kunchandy et al.[16].

2.6. Estimation of gastric secretion parameters and histology of the tissue

Protective index was determined using the method of Hano et al.[17].

\[
\text{Protective} \% = \frac{\text{Ulcer control} - \text{Pretreated group} (\text{UI})}{\text{Ulcer control} (\text{UI})} \times 100
\]

Free and total acidity was determined by method of Hawk et al.[18].

\[
\text{Acidity} = \frac{\text{Volume of NaOH} \times \text{Normality of NaOH}}{0.1} \times 100
\]

Total protein content was estimated following the principle described by Tietz[19], pepsin activity was estimated by the method of Debnath et al.[20] and mucin concentration was determined by the method described by Dische and Shettles[25], and sialic acid concentration was determined by the method described by Warren[26].

2.7. Determination of total carbohydrates

The dissolved mucosubstance in the gastric juice was used for the estimation of total carbohydrate by the method described by Goel et al.[22]. The method of Winzler[23] was used for the determination of hexoses, whilst hexosamine by the method of Disch and Borenfreund[24]. Fucose concentration was determined by the method described by Dische and Shettles[25], and sialic acid concentration was determined by the method described by Warren[26].

2.8. Isolation and estimation of mitochondrial functional parameters

Isolation of stomach mitochondrial fraction was carried out according to the method described by Schneider and Hogboom[27]. Succinate dehydrogenase activity was assessed according to the method of Slater and Bonner[28]. The activity of aconitase was estimated according to the method of Kenneddy et al.[29]. Total ATPase activity in the stomach mitochondrial was determined by the method of Evans[30]. The activity of Ca²⁺-ATPase present in the isolated mitochondrial fraction was assayed according to the method of Hjerten and Pan[31]. Mg²⁺-ATPase present in the isolated mitochondrial fraction was assayed according to the principle described by Ohnishi et al.[32]. Na⁺/K⁺-ATPase was estimated according to the principle described by Bonting[33]. The amount of phosphorus liberated was estimated according to the method of Fiske and Subbarow[34]. Mitochondrial swelling was estimated by monitoring the changes in absorbance at 520 nm spectrophotometry as described by Halestrap and Davidson[35]. Nicotinamidadenine dinucleotide-reduced (NADH) level in the mitochondria was determined according to the method of Minezaki et al.[36]. Mitochondrial membrane potential (MMP) was determined according to the principle described by Emaus et al.[37].
2.9. Statistical analysis

Results obtained in this study were expressed as the mean ± SEM. Statistical significance of difference in parameters was determined by One-way analysis of variance and Duncan’s multiple range test using SPSS software version 22. P < 0.05 was considered to be significant.

3. Results

Confirmatory test for the presence of tannin gave dark-green colour when the extracted phytochemical was treated with ferric chloride, which confirms the presence of tannin. The percentage of polyphenol content contained in the extract was found to be 30% and the percentage of tannin in the crude extract was 60%. The experimental results in Table 1 show that the activities of mitochondrial ATPase were low in Group II (untreated) compared to pretreated groups. Administration of tannin and omeprazole boosted the activities of these mitochondrial enzymes. In Table 2, there was a reduction in the level of fucose, sialic acid, hexosamine and hexose in Group II compared to pretreated groups. The activities of TCA cycle enzymes (succinate dehydrogenase and aconitase) in Group II were low compared to control and pretreated groups, and significantly high in rats pretreated with different doses of tannin and omeprazole as shown in Table 3. In Table 4, we observed that there was a drastic increase in stomach acid in Group II compared to rats pretreated with tannin. In Table 5, different doses of the extracted tannin reduced the protein content of the gastric juice. The activity of pepsin in Group II (untreated) was extremely high, on the other hand, administrations of the extracted tannin significantly reduced pepsin activity. There was a significant drop in mucin level in Group II compared to pretreated groups and non significant between control and rats pretreated with 200 mg/kg body weight of tannin. In Table 6, Group VI had the highest value for calculated ulcer protective index (75%) followed by Groups V and IV, while Group III had the least ulcer protective index.

In Figure 2, there was a decrease in gastric tissue mitochondrial absorbance in Group II (untreated), demonstrating stomach dysfunction through administration of aspirin. The absorbance of pretreated groups was significantly increased, indicating that tannin from S. americana improved impaired stomach mitochondrial functions.

Figure 3 shows that there was loss of mitochondrial membrane potential in Group II, while tannin from S. americana and omeprazole significantly enhanced mitochondrial function. In Figure 4, we also observed significant decreases in NADH level in stomach mitochondrial in Group II (untreated), when compared to the control group. This indicates that extracted tannin significantly increased NADH level, demonstrating that tannin from S. americana enhanced cellular stress response capacity.

In Figure 5, normal mucosa is shown in Group I, whilst patchy irregularly shaped ulcer is shown in Group II. In the pretreated groups, funnel and focal shaped ulcer is shown. The protective effect of tannin on gastric mucosa increased in dose dependent manner.

Table 1

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Doses mg/kg body weight</th>
<th>Ca&lt;sup&gt;2+&lt;/sup&gt; ATPase (units/mL)</th>
<th>Na&lt;sup&gt;+&lt;/sup&gt;/K&lt;sup&gt;+&lt;/sup&gt; ATPase (units/mL)</th>
<th>Mg&lt;sup&gt;2+&lt;/sup&gt; ATPase (units/mL)</th>
<th>Total ATPASE (units/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Water</td>
<td>1 mL</td>
<td>23.63 ± 1.09</td>
<td>9.31 ± 0.48</td>
<td>10.21 ± 0.49</td>
<td>46.55 ± 0.36</td>
<td></td>
</tr>
<tr>
<td>II Water</td>
<td>1 mL</td>
<td>12.61 ± 2.11</td>
<td>3.90 ± 0.09</td>
<td>4.04 ± 0.03</td>
<td>31.04 ± 0.54</td>
<td></td>
</tr>
<tr>
<td>III Tannin 50</td>
<td>17.05 ± 0.62</td>
<td>5.94 ± 0.20</td>
<td>6.22 ± 0.34</td>
<td>37.18 ± 0.36</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV Tannin 100</td>
<td>19.62 ± 0.44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.51 ± 0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.36 ± 0.55</td>
<td>37.54 ± 1.87&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V Tannin 200</td>
<td>20.91 ± 0.67&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.09 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.28 ± 0.41&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>45.10 ± 1.91&lt;sup&gt;e&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VI Omeprazole 20</td>
<td>21.84 ± 0.92&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>8.76 ± 0.19&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>9.74 ± 0.90&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>45.48 ± 1.26&lt;sup&gt;cd&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are given as means ± SEM for 6 rats in each group. Values sharing a different superscript vertically differ significantly at P < 0.05.

Table 2

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatments</th>
<th>Doses mg/kg body weight</th>
<th>Hexasse (µg/mL)</th>
<th>Fucose (µg/mL)</th>
<th>Sialic Acid (µg/mL)</th>
<th>Hexosamine (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Water</td>
<td>1 mL</td>
<td>28.90 ± 0.13</td>
<td>123.35 ± 0.22</td>
<td>54.44 ± 0.32</td>
<td>20.50 ± 0.76</td>
<td></td>
</tr>
<tr>
<td>II Water</td>
<td>1 mL</td>
<td>12.42 ± 0.14</td>
<td>31.52 ± 0.61&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.78 ± 1.42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.50 ± 0.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>III Tannin 50</td>
<td>17.38 ± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>92.13 ± 1.67&lt;sup&gt;b&lt;/sup&gt;</td>
<td>32.30 ± 0.36&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.50 ± 0.28&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV Tannin 100</td>
<td>17.62 ± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>94.28 ± 0.48&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>32.78 ± 0.32&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>7.20 ± 0.35&lt;sup&gt;cd&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V Tannin 200</td>
<td>27.29 ± 0.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>115.14 ± 1.67&lt;sup&gt;e&lt;/sup&gt;</td>
<td>43.92 ± 0.43&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>16.80 ± 1.01&lt;sup&gt;e&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VI Omeprazole 20</td>
<td>27.95 ± 0.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>122.91 ± 0.11&lt;sup&gt;e&lt;/sup&gt;</td>
<td>49.08 ± 0.07&lt;sup&gt;e&lt;/sup&gt;</td>
<td>17.83 ± 0.44&lt;sup&gt;e&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are given as means ± SEM for 6 rats in each group. Values sharing a different superscript vertically differ significantly at P < 0.05.

Table 3

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatments</th>
<th>Doses in mg/kg body weight</th>
<th>SDH (unit/mL)</th>
<th>Aconitase (millimils/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Water</td>
<td>1 mL</td>
<td>1.07 ± 0.18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.14 ± 0.27&lt;sup&gt;e&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>II Water</td>
<td>1 mL</td>
<td>0.27 ± 0.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.10 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>III Tannin 50</td>
<td>0.53 ± 0.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.27 ± 0.15&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV Tannin 100</td>
<td>0.67 ± 0.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.38 ± 0.11&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V Tannin 200</td>
<td>0.80 ± 0.12&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.70 ± 0.32&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VI Omeprazole 20</td>
<td>1.00 ± 0.12&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.04 ± 0.24&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are given as means ± SEM for 6 rats in each group. Values sharing a different superscript vertically differ significantly at P < 0.05.

Table 4

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Doses in mg/kg body weight</th>
<th>pH</th>
<th>Volume of gastric juice (mL)</th>
<th>Free Acidity (mEq/L)</th>
<th>Total Acidity (mEq/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Water</td>
<td>1 mL</td>
<td>5.08 ± 0.29&lt;sup&gt;b&lt;/sup&gt; 2.39 ± 0.25&lt;sup&gt;c&lt;/sup&gt;</td>
<td>46.25 ± 0.48</td>
<td>94.75 ± 0.25&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>II Water</td>
<td>1 mL</td>
<td>5.20 ± 0.07&lt;sup&gt;e&lt;/sup&gt; 4.00 ± 0.18&lt;sup&gt;e&lt;/sup&gt;</td>
<td>70.50 ± 0.50&lt;sup&gt;e&lt;/sup&gt;</td>
<td>131.25 ± 0.25&lt;sup&gt;e&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III Tannin 50</td>
<td>4.83 ± 0.10&lt;sup&gt;e&lt;/sup&gt; 4.10 ± 0.07&lt;sup&gt;e&lt;/sup&gt;</td>
<td>58.25 ± 0.65&lt;sup&gt;e&lt;/sup&gt;</td>
<td>111.25 ± 0.25&lt;sup&gt;e&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV Tannin 100</td>
<td>5.13 ± 0.10&lt;sup&gt;e&lt;/sup&gt; 3.60 ± 0.22&lt;sup&gt;e&lt;/sup&gt;</td>
<td>51.75 ± 0.25&lt;sup&gt;e&lt;/sup&gt;</td>
<td>107.50 ± 0.29&lt;sup&gt;e&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V Tannin 200</td>
<td>4.99 ± 0.11&lt;sup&gt;e&lt;/sup&gt; 3.33 ± 0.13&lt;sup&gt;e&lt;/sup&gt;</td>
<td>48.75 ± 0.75&lt;sup&gt;e&lt;/sup&gt;</td>
<td>100.00 ± 0.41&lt;sup&gt;ef&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VI Omeprazole 20</td>
<td>5.18 ± 0.10&lt;sup&gt;ef&lt;/sup&gt; 3.15 ± 0.10&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>49.00 ± 0.71&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>99.25 ± 0.55&lt;sup&gt;ef&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are given as means ± SEM for 6 rats in each group. Values sharing a different superscript vertically differ significantly at P < 0.05.
Table 5

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Doses in mg/kg body weight</th>
<th>Protein (g/dL)</th>
<th>Pepsin (µg/mL)</th>
<th>Mucin (mg hexose %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Water</td>
<td>1 mL</td>
<td>1.55 ± 0.02a</td>
<td>6.29 ± 0.16a</td>
<td>47.13 ± 1.22d</td>
</tr>
<tr>
<td>II</td>
<td>Water</td>
<td>1 mL</td>
<td>4.22 ± 0.02b</td>
<td>13.78 ± 0.18a</td>
<td>22.95 ± 1.29f</td>
</tr>
<tr>
<td>III</td>
<td>Tannin</td>
<td>50</td>
<td>2.74 ± 0.06d</td>
<td>9.28 ± 0.18d</td>
<td>33.35 ± 0.81c</td>
</tr>
<tr>
<td>IV</td>
<td>Tannin</td>
<td>100</td>
<td>2.31 ± 0.23c</td>
<td>8.40 ± 0.03c</td>
<td>35.43 ± 1.51d</td>
</tr>
<tr>
<td>V</td>
<td>Tannin</td>
<td>200</td>
<td>1.63 ± 0.03b</td>
<td>6.73 ± 0.10b</td>
<td>43.53 ± 1.22d</td>
</tr>
<tr>
<td>VI</td>
<td>Omeprazole</td>
<td>20</td>
<td>1.57 ± 0.03a</td>
<td>6.59 ± 0.23ab</td>
<td>44.08 ± 0.95d</td>
</tr>
</tbody>
</table>

Values are given as means ± SEM for 6 rats in each group. Values sharing a different superscript vertically differ significantly at $P < 0.05$.

Table 6

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatments</th>
<th>% Protective</th>
<th>Ulcer index</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (Control)</td>
<td>Water</td>
<td>–</td>
<td>0.00 ± 0.00a</td>
</tr>
<tr>
<td>II (Ulcerated)</td>
<td>Water</td>
<td>–</td>
<td>13.88 ± 0.15d</td>
</tr>
<tr>
<td>III (Tan 50)</td>
<td>Tannin</td>
<td>52</td>
<td>7.03 ± 0.09e</td>
</tr>
<tr>
<td>IV (Tan 100)</td>
<td>Tannin</td>
<td>62</td>
<td>6.33 ± 0.19b</td>
</tr>
<tr>
<td>V (Tan 200)</td>
<td>Tannin</td>
<td>72</td>
<td>3.40 ± 0.23c</td>
</tr>
<tr>
<td>VI (20)</td>
<td>Omeprazole</td>
<td>75</td>
<td>2.98 ± 0.09a</td>
</tr>
</tbody>
</table>

Administration of tannin and standard antiulcer drug (omeprazole) indicated a significant decrease ($P < 0.05$) in ulcer index when compared to Group II. Values are given as means ± SEM for 6 rats in each group. Values sharing a different superscript vertically differ significantly at $P < 0.05$.

Figure 2. The effect of tannin from *S. americana* plant on mitochondrial swelling.

Mitochondria were isolated from rat stomach subjected to aspirin and those pretreated with different doses of tannin, and were incubated in reaction buffer containing 250 mmol/L sucrose, 0.3 mmol/L CaCl₂ and 10 mmol/L Tris (pH 7.4). Mitochondrial swelling was monitored by measuring the change in absorbance at 540 nm ($\Delta A_{540}$). The absorbance at 540 nm in Group I (control) mitochondria declined significantly compared to Group II (ulcerated rats). This indicates that there was mitochondrial dysfunction in rat stomach mitochondrial by administration of aspirin. But prior administration of tannin and omeprazole increased the absorbance signifying improvement in damaged mitochondrial function.

![Figure 2](image-url)

Figure 3. Protective effect of tannin from *S. americana* plant on mitochondrial membrane potential dissipation induced by aspirin.

There was loss of mitochondrial membrane potential in Group II (ulcerated rats) compared with Group I (normal rats), while tannin from *S. americana* and omeprazole significantly enhanced mitochondrial function. Values are given as means ± SEM for 6 rats in each group. $^a$: $P < 0.05$ compared with the ulcerated group.

![Figure 3](image-url)

Figure 4. NADH Level in groups pretreated with different doses of tannin and omeprazole in aspirin induced gastric ulcer.

Values are given as means ± SEM for 6 rats in each group. $^a$: $P < 0.05$ compared with the ulcerated group.

![Figure 4](image-url)

Figure 5. Micrographs of histological section of rats stomach in various Groups I-VI.

Group I (control) shows normal mucosa; Group II, ulcerated rats stomach shows patchy irregularly shaped ulcer; Group III, rats pretreated with 50 mg/kg of tannin shows funnel shaped ulcer; Group IV, the rat stomach pretreated with 100 mg/kg body weight of tannin shows a superficial erosion; Group V, mild mucosal congestion, rat stomach given 200 mg/kg of tannin shows focal irregularly shaped ulceration; Group VI, rat stomach pretreated with omeprazole shows focal funnel shaped ulcer and muscularis mucosa.

![Figure 5](image-url)
4. Discussion

Tannin from *S. americana* was investigated for its gastroprotective effect in ulcerogenic rats. The different doses of tannin extract (50 mg/kg, 100 mg/kg and 200 mg/kg) used in this study significantly reduced the activity of pepsin, quantity of gastric juice and acid secretion compared to ulcerogenic group (P < 0.05). Over secretion of stomach acid is the major cause of gastric and duodenal ulcers, termed ‘aggressive factor’. The antiulcer activity of tannin from *S. americana* may be due to its ability to reduce the secretion of stomach acid. Nonsteroidal anti-inflammatory drugs such as aspirin used in this study cause mucosal injury by inhibiting prostaglandin synthesis which results to increased secretion of stomach acid. Prostaglandins protect the gastric mucosa by increasing the secretion of bicarbonate ion which neutralizes the acidity of the stomach. It has been reported that inhibition of acid secretion is the major factor responsible for the healing of gastric ulcer. Aspirin inhibits cyclooxygenase the enzyme responsible for the synthesis of prostaglandin and also irritates the gastric mucosa. In addition, prostaglandin acts as cytoprotective role on the gastrointestinal epithelium which enhances the integrity of gastric mucosa. Extremely low pH can lead to the development of gastrointestinal diseases. Studies have also shown that decrease in the level of hydrogen ion in the gastric juice is due to low pH which is responsible for the development of gastrointestinal disease. The effects noticed for pH value from the study, when ulcerogenic rats were compared to pretreated rats may be due to gastroprotective attributes of tannin extract used in this study.

There was significant (P < 0.05) increase in ulcer index in Group II compared to pretreated rats. On the other hand, pretreatments with different doses of tannin significantly reduced ulcer index which might be attributed to increased prostaglandin synthesis. Experimental evidences have also shown that inhibition of prostaglandin synthesis reduced mucosal blood flow and damage the gastric mucosa.

The outside layer of the stomach is hydrophobic which can be reduced by different chemicals and drugs such as aspirin. It has been reported that inhibition of mucus and bicarbonate secretion by aspirin reduces the hydrophobicity of the outer layer of the stomach. Decreased noticed in mucin concentration in Group II may be due to distorted hydrophobicity of the mucous in the stomach which reduced the ability of the mucosal membrane to protect the mucosa against various aggressive factors such as aspirin. Pretreatment with tannin elevated mucin level in the gastric juice, this is an indication of improved mucus secretory potential which plays a significant role in gastroprotective process. Prostaglandins play a vital role in mucosal defense by stimulating mucus and secretion of bicarbonate. Mucus also slows down the distribution of stomach acid, and secretion of bicarbonate neutralises it. Aspirin reduces mucus secretion, its viscosity and mucus glycoproteins biosynthesis. This is in agreement with the presence work that was administration of aspirin decreased mucin concentration but prior administration of tannin elevated mucin content.

Administration of tannin and omeprazole prevented plasma protein from escaping into the gastric juice. We observed that there was significant (P < 0.05) increased in protein concentration in Group II compared to pretreated groups. The low level of protein found in the gastric juice indicated that there was a decrease in plasma protein that escaped into the gastric juice. High level of protein found in the gastric juice in Group II suggested mucosa injury while tannin from *S. americana* fortified the gastric mucosa against aspirin induced gastric damage.

Oxidative phosphorylation is a process by which mitochondria couples respiration to generate ATP. Experimental evidence suggested that mucosa injury may be initiated by the action of NSAIDs on mitochondria. NSAIDs inhibit mitochondrial oxidative phosphorylation, decreased ATP synthesis and dissipate mitochondrial transmembrane potential. Significant (P < 0.05) decreased in the activity of succinate dehydrogenase and aconitase observed in Group II suggested that there was distortion of mitochondrial energy coupling process. Administration of tannin maintained the level of mitochondrial ATPases and activities of TCA cycles enzymes which reflects the ability of extracted tannin from *S. americana* to enhance oxidative phosphorylation for the generation of ATP.

Percentage protection against aspirin induced ulcer in this study increased in dose dependent manner showing high protection by 200 mg/kg body weight of tannin than 50 mg/kg body weight, but the percentage protection of the omeprazole was found to be the highest among all the groups, thus revealing the protective role of tannin from *S. americana* against gastric mucosal lesions in aspirin induced ulceration in rats.

There was significant (P < 0.05) decrease in mitochondrial absorbance in Group II compared to control group, signifying that administration of aspirin induced stomach mitochondrial dysfunction. We noticed that there was an increase in the absorbance of pretreated groups, indicating that tannin from *S. americana* improved impaired stomach mitochondrial functions. There was a decrease in NADH level in Group II compared to pretreated groups, extracted tannin significantly increased NADH level, demonstrating its ability to maintain mitochondrial energy metabolism.

The loss of mitochondrial membrane potential is an indication of mitochondrial injury. Administration of aspirin in this study induces mitochondrial dysfunction demonstrated by loss of mitochondrial membrane potential. Tannin from *S. americana* significantly improved impairment in mitochondrial function induced by aspirin.

Histomorphological evaluation of stomach shows that the general gastric morphology was maintained in control and treatment with graded doses of tannins as well as omeprazole for 7 days before aspirin had comparable attenuating effects on the ulcerated gastric mucosa producing focal (localized) areas and superficial erosion. The highest dose of tannin (200 mg/kg body weight) used in this study and omeprazole gave a better protection.

Tannin from *S. americana* can thus be considered as a cytoprotective agent probably due to decreased acid secretion and increased mucus secretion which protect the gastric mucosa against various chemicals and drugs. It also prevented damage of stomach mitochondrial functions by restoring the TCA cycle enzymes and ATPases. Therefore tannin from *S. americana* may be consider as another alternative source for the treatment of gastric ulcer.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

We expressed our gratitude to Mr. Philip Iorliam for supply of the *Schwenkia americana* Linn. plant. We also thank Dr. Akinnibosun Henry (Herbarium Unit University of Benin, Nigeria) for authentication of the *Schwenkia americana* Linn. plant.

References


