Protective effects of *Dialium guineense* pulp on aspirin-induced gastric mucosal injury in albino rats

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**Abstract**

The numerous challenges and detrimental effects connected with the treatment of peptic ulcers in the world today calls for alternative attention. Ethnomedicinally, *Dialium guineense* pulp (DAGP) has numerous pharmacological activities. This study investigated the anti-ulcer activities of *Dialium guineense* pulp on gastric mucosa injury induced with aspirin in albino Wistar rats. DAGP extract was orally administered at doses of 250, 500 and 1000 mg/kg bw (mg per kg of the body weight) per day for 3 or 7 days followed by 400 mg/kg bw oral aspirin administration. Ulcer indices were determined, followed by a biochemical estimation of antioxidant enzymes using gastric mucosal tissue from the stomach. Student’s *t*-test was used to compare significant differences among groups of animals at *P* ≤ 0.05. The results showed that *Dialium guineense* pulp caused a significant decrease (*P* ≤ 0.05) in the ulcer index in aspirin induced rats. This decrease in ulcer index is dose dependent and 1000 mg/kg bw per day caused the highest decrease in 7 days. The results showed a significant increase (*P* ≤ 0.05) in lipid peroxidation and a decrease (*P* ≤ 0.05) in antioxidant enzymes activities in the aspirin-induced ulcerated rats. Oral administration of DAGP increased antioxidant enzymes activities and decreased injury in the gastric mucosa in ulcer induced rats. Therefore, this study showed that DAGP exhibited anti-ulcer potential and that the gastrointestinal protection may be through the scavenging action of free radicals by its constituent antioxidants. Thus, *Dialium guineense* pulp has ameliorative medicinal potential for the curing of gastric disorders.

**Keywords:** Antioxidant enzymes; Aspirin; *Dialium guineense* pulp (DAGP); Ethnomedicine; Gastric ulcer

**Highlights:**
- *Dialium guineense* pulp (DAGP) extract exhibits cyto-protection in gastric ulcer.
- The extract exhibited significant antioxidant activities and reduces cellular oxidative stress through its free radical scavenging potentials.
- *Dialium guineense* pulp (DAGP) extract reduces lipid peroxidation and thus ultimately reduces loss of cellular functions.
- *Dialium guineense* pulp (DAGP) extracts can improve health of ulcer subjects.

**Introduction**

Peptic ulcer is a medical condition involving a group of chronic, non-malignant inflammatory disorders characterized by the presence of mucosal damage leading to the formation of a sore area in the stomach and duodenum where parietal (acid-secreting) cells are located (Al Batran et al., 2013; Wasman et al., 2010). The etiology of peptic ulcer is multifactorial, ranging from *Helicobacter pylori* infection (Sumbul et al., 2011; Verma, 2010) to antiplatelet agents (Yeomans et al., 2009), non-steroidal anti-inflammatory drugs (Lanas, 2009; Søberg et al., 2010), serotonin reuptake inhibitors (Itatsu et al., 2011), excessive alcohol consumption and cigarette smoking, among others (Seberg et al., 2010). These factors can expose the upper gastro-intestinal tract to an excessive amount of acid (Abdel-lah et al., 2010; Verma, 2010), submucosal erosion, and inhibit cyclooxygenase needed for gastric mucosa layer protection (Paiotti et al., 2012).

The pathological processes involved in gastrointestinal disorders have direct links with agents that induce oxidative stress and mediate free radical processes (Garrow and Delegge, 2010; Salim, 1992). Aspirin is a potent therapeutic treatment belonging to the non-steroidal anti-inflammatory drugs (NSAIDs). Reports showed that aspirin causes necrotic lesions in the gastrointestinal mucosa (Konturek et al., 1999). The...
necrotic lesions, which are usually produced in multiple pathways, reduce defensive factors via the induction of oxidative stress (Dursun et al., 2009; Lanas, 2009). Research evidence showed that long term use or abuse of aspirin causes peptic ulcer via the increase and aggravation of oxidative stress, gastric acid and pepsin secretions, gastric microcirculation and prostaglandin E2 content in gastrointestinal mucosa cells (Laine et al., 2008). Reactive oxygen species cause damage to gastrointestinal mucosa and thus, the pathogenesis of aspirin-induced erosive gastritis may be due to the free oxygen-radicals (Sumbul et al., 2011).

Peptic ulcer affects about 5–10% of the world population within the span of their life-time (Garrow and Delegge, 2010) and the synthetic drugs utilized for its treatment have numerous adverse effects in humans. Currently, plants widely applied for the prevention and treatment of well-known diseases are constantly evolving in the developing world since they have no adverse health effects (Mahmood et al., 2011; Obasi et al., 2013). Plants contain bioactive compounds with potentials for effective treatment of diseases such as cancer, rheumatism and inflammatory diseases, etc. (Atawodi, 2005; Bardi et al., 2011). The curative potentials of these plants serve as catalysts for exploring and probing their activities, especially the commonly wild underutilized medicinal plants (Mahmood et al., 2011; Obasi et al., 2013).

\textit{Dialium guineense}, popularly called velvet tamarind, is a tall tree common in the tropics which bears fruit and belongs to the Leguminosae family. The fruit is edible, the size of a small grape and has a brown hard shell. In Africa, especially along the southern edge of the Sahel and West African countries, the tree grows in dense forests (Dressler et al., 2014). The fruit has one shiny, brown-coloured, flat-rounded hard seed. The seed measures 7–8 millimetres wide and 3 millimetres thick. The edible portion of the fruit is the pulp which can be eaten in raw form or processed into a consumable beverage.

The food and medicinal values of \textit{Dialium guineense} is well documented. Its medicinal potentials – including antimicrobial, antioxidant, analgesic, anti-hepatotoxic and anti-plasmodial, among others – are well documented (Adeleye et al., 2014; Adumanya et al., 2013; Balogun et al., 2013; Besong et al., 2016; Ezeja et al., 2011; Orji et al., 2012).

Therefore, this study was designed and carried out to evaluate the antiulcerogenic effects of \textit{Dialium guineense} pulp (DAGP) on aspirin-induced damage in gastric mucosa through its effect on the antioxidative enzymes.

\section*{Materials and methods}

\subsection*{Collection and identification of plant materials}

The ripe fruits (Fig. 1) were collected from the \textit{Dialium guineense} plant tree at Enyum Otu-Ukpa, Akpoha in Afikpo North, Ebonyi State, Nigeria with coordinates, latitude 5° 22' 45" North and longitude 7° 42' 30" East. The samples were identified and authenticated by the Polytechnic plant curator at the Biology Research Unit, Department of Science Laboratory Technology, Akanu Ibiam Federal Polytechnic Unwana, Nigeria. The spoilt and bruised fruits were removed and the acceptable fruits were thoroughly washed with tap water to remove debris. Thereafter, the selected fruits were air-dried. The pulp was then separated from the seed and shell and was shade dried in the open air for three weeks. The air-dried pulp samples were then grounded into a coarse powder using a manual milling machine. The powdered samples were stored in airtight containers under cool and dry conditions until required for further analysis.

\begin{figure}[!h]
\centering
\includegraphics[width=0.7\textwidth]{fig1.jpg}
\caption{\textit{Dialium guineense} fruits (A) Unshelled \textit{Dialium guineense} fruits with the stalk on the tree, (B) Harvested unshelled \textit{Dialium guineense} fruits with the seed coat and (C) Shelled \textit{Dialium guineense} fruits with seed coats removed exposing the edible portion used for the study.}
\end{figure}
**Aspirin (Acetyl salicylic acid, Aspegic)**
Aspirin tabulate (500 mg) manufactured by Bayer Schering Pharma AG, Germany, was bought from a licensed pharmacy (Good Health Pharmacy, Afikpo, Nigeria).

**Preparation of the sample extract**
Exactly 200 g of the processed pulp samples were placed into a container and 500 ml of methanol was added as extracting solvent. The mixture was soaked for 72 h with intermittent shaking. Thereafter, the mixture was filtered using Whatman No. 42 filter paper. The filtrate collected was then distilled using a rotary evaporator (Eyela™, USA). The extract was cryo-desiccated in a freeze-dryer for the production of the dried powdered extract (yield: 19.5%) and it was stored at −4 °C until needed for use (Al Batran et al., 2013; Mahmood et al., 2011). The extract was lyophilized with distilled water and administered via oral intubation to the various groups of the albino Wistar rats at graded dosages (250, 500 and 1000) mg/kg body weight.

**Experimental animals**
Male albino Wistar rats weighing 150–200 g and belonging to the same age bracket were obtained and used throughout the experiments. The rats were sourced from the University of Nigeria, Nsukka (animal facility units). The albino Wistar rats were housed in a wooden cage in the animal house of the Biology Research Unit of Akanu Ibiam Federal Polytechnic Uwana under standard conditions of temperature and humidity. They were allowed access to clean water ad libitum and fed with rat chow (Grower Pellet of Chikun Feeds, Kaduna; for feed composition see Suppl. Table S1) and maintained in a 12 h day and 12 h night cycle. Experiments involving all of the animals were done in line with the Institutional Animal Ethics Committee guidelines, as adopted by Alex Ekwueme Federal University (AEFUNAI) College of Medical Sciences Research Ethics Committee. This research was approved by the Committee in line with their mandate with reference number 18/AEFUNAI/COM/R/VOL.1/032.

**Dosage and treatment**

**Acute toxicity study**
In this study, median lethal dose (LD₅₀) of the methanolic extract of *Dialium guineense* pulp was evaluated by Lorke (1983) method. In the prospective study, nine male albino Wistar rats were used. The albino Wistar rats were assigned at random to three groups (A, B, and C) comprising three (3) rats in each group. The methanolic *Dialium guineense* pulp extract was then administrated through intra-peritoneal injection to group A, B and C at doses of 10, 100 and 1000 mg/kg bw (mg per kg of the body weight), respectively. The albino Wistar rats were then monitored for any signs of behavioral changes and mortality for 24 h. After 24 h, there was no mortality in the treated groups. In order to confirm the safety of the extract as to inform the oral dosage for use, another set of albino Wistar rats were randomly assigned to five other groups (I to V) and each group were treated with 1250, 1500, 2000, 2500 and 5000 mg/kg bw, of the DAGP extract, respectively (through oral administration). These rats were then monitored for 24 h for any signs of behavioral changes and mortality. The geometric means of the lowest dosage that killed and the highest dosage that did not kill an albino Wistar rat were used to calculate the median lethal dose (LD₅₀) of the DAGP extracts for the albino Wistar rats.

**Experimental design and procedure**
The experiment was conducted in two stages called Phase I and Phase II. In Phase I, the *Dialium guineense* pulp (DAGP) was administered orally at doses of 250, 500 and 1000 mg/kg bw per-day as a distilled water prepared aqueous suspension at timed intervals for 3 or 7 days respectively (for data on body weight change and daily food intake see Suppl. Table S2). Thereafter the experiment, 400 mg/kg bw of aspirin suspended in 0.5% carboxy methyl cellulose was administered orally to the albino Wistar (Jaarin et al., 2002).

**PHASE I**
Group A – Albino Wistar rats received 400 mg/kg bw of aspirin + pre-treatment with 250 mg/kg bw per day of DAGP administered orally for 3 days.

Group B₁ – Albino Wistar rats received 200 mg/kg bw of aspirin + pre-treatment with 250 mg/kg bw per day of DAGP administered orally for 7 days.

Group B₂ – Albino Wistar rats received 400 mg/kg bw of aspirin + pre-treatment with 500 mg/kg bw per day of DAGP administered orally for 7 days.

Group B₃ – Albino Wistar rats received 400 mg/kg bw of aspirin + pre-treatment with 1000 mg/kg bw per day of DAGP administered orally for 7 days.

Group C₂ – Albino Wistar rats received 400 mg/kg bw of aspirin + pre-treatment with 500 mg/kg bw per day of DAGP administered orally for 7 days.

Group D – Albino Wistar rats received 400 mg/kg bw of aspirin + pre-treatment with 1000 mg/kg bw per day of DAGP administered orally for 7 days.

Group D₂ – Albino Wistar rats received 400 mg/kg bw of aspirin + pre-treatment with 1000 mg/kg bw per day of DAGP administered orally for 7 days.

**Gastric ulcer index:** Agrawal et al. (2000) outlined the method adopted and used for this study. The albino Wistar rats were subjected to Diethyl ether overdose for scarification after 4 h of aspirin administration, and their stomachs were removed and washed with saline. The stomachs were incised through the greater curvature and washed with saline and used to determine the gastric lesions in the gastric glandular portion using a dissecting microscope (Agrawal et al., 2000; Al Batran et al., 2013). The ulcer index was obtained by summing the lengths of all measured lesion areas for each albino Wistar rat. The curative ratio for each group was calculated using equation (i)

\[
Curative\ ratio\ (CR) = \frac{(LC - LT/LC)}{100}
\]

Where:
- **LC:** The length of gastric ulcer in the control group.
- **LT:** The length of gastric ulcer in the treated group.

From the study done in Phase I, a dosage of 1000 mg/kg bw per day of DAGP administered orally for 7 days appears more efficacious in preventing gastric lesions induced by the orally administered aspirin. Therefore, this 1000 mg/kg bw per day of DAGP was selected as an effective dosage to carry out further studies in Phase II.

The rats were divided into four groups of six each. The animals were kept fasting for 48 h prior to the experiment but had access to water ad libitum.
**PHASE II**

Group I – Normal control.

Group II – Albino Wistar rats received 400 mg/kg bw of aspirin suspended in 0.5% carboxy methyl cellulose via oral administration (Negative control).

Group III – Albino Wistar rats received 400 mg/kg bw of aspirin + pre-treatment with 1000 mg/kg bw per day of DAGP administered orally for 7 days.

Group IV – Albino Wistar rats received 1000 mg/kg bw per day of DAGP administered orally for 7 days.

**Biochemical estimation**

The gastric mucosal tissue obtained from the initial portion of the pyloric part of the stomach was used for the biochemical estimation. The mucosal homogenate was prepared by scraping the gastric mucosa using a scraper to obtain a gastric mucosal homogenate which was weighed and homogenized in ice-cold phosphate buffer (pH 7.2). Thereafter, the homogenate was centrifuged for 10 min at 3000 rpm to obtain the supernatant, used for the studies. The method outlined in Lowry et al. (1951) was used to estimate the protein content using a standard (Bovine serum albumin). Lipid peroxidation products estimation was done using the method outlined by Das et al. (1994) by assaying malondialdehyde formation. Ellman (1959) method was used to estimate the reduced glutathione level (total tissue sulphydryl -thiolS group) while the method outlined by Misra and Fridovich (1972) was employed to determine the activities of superoxide dismutase (SOD) taking into cognizance epinephrine autooxidation. The activities of catalase (CAT) were determined by the method of Beers and
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PHASE II

Fig. 3. Overview of experimental design Phase II

Results

The methanol extract of Dialium guineense pulp (DAGP) yielded 19.5% dry weight. The results showed that no death of an albino Wistar rats orally treated with Dialium guineense pulp extract at doses 1250, 1500, 2000, 2500 and 5000 mg/kg bw was recorded during the acute toxicity study. The DAGP extract was well tolerated by the rats without any overt signs of behavioral changes as a result of possible toxicity.

The study findings on the effects of Dialium guineense pulp methanol extract on gastric lesions induced by aspirin in albino Wistar rats are presented in Table 1.

Statistical analysis

The results obtained were expressed as mean ± standard error of the mean (SEM) of six replicates. The results were analyzed using SPSS Windows version 21.0 (SPSS, Chicago, IL) and the means were compared between different groups. Student’s t-test was used to assess statistical significant differences in various groups of the albino Wistar rats at P < 0.05.

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The results (Table 1) showed that oral administration of DAGP doses at 250, 500 and 1000 mg/kg bw decreased ulcer index in aspirin induced albino Wistar rats in a dose dependent manner. The results also revealed that the dose of 1000 mg/kg showed marked lesion inhibition with maximum ulcer protection of 84.64% at 7 days of treatment with the extract. Since this dosage inhibited the lesions with very high ulcer protection without the albino Wistar rats exhibiting any symptoms of overt toxicity, it means that the extract at this dose is safe and efficacious and thus was employed for biochemical studies in phase II.

The results of the gastric lesions, protein levels, lipid peroxidation and antioxidant enzymes in gastric mucosa of albino Wistar rats are presented in Table 2.

The results in Table 2 showed a significant decrease ($P < 0.05$) in the protein levels and SOD, CAT, GPX, GSH and GST activities in Group II (Albino Wistar rats received 400 mg/kg-bw of aspirin suspended in 0.5% carboxy methyl cellulose via oral administration (Negative control)) compared to Group I (Normal control). Also, a significant increase ($P < 0.05$) in lipid peroxide (LPO) activities was observed in Group II as compared to Group I. Also, the results showed that no significant difference ($P > 0.05$) existed in the activities of LPO, CAT and GPX in Group IV (Albino Wistar rats received 1000 mg/kg bw per day of DAGP administered orally for 7 days) as compared to Group I but a significant increase ($P < 0.05$) existed in the levels of protein and activities of SOD, GSH and GST enzymes in Group IV as compared to Group I (Table 2). The results (Table 2) also indicated that LPO activities significantly decreased ($P < 0.05$) in Group III (Albino Wistar rats received 400 mg/kg bw of aspirin + pre-treatment with 1000 mg/kg bw per day of DAGP administered orally for 7 days) when compared to Group II, while the levels of protein and activities of SOD, CAT, GPX, GSH and GST in Group III increased significantly ($P < 0.05$) as compared to Group II.

### Table 1. Effects of *Dialium guineense* pulp (DAGP) on lesions scores in aspirin-induced gastric ulcer in albino Wistar rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment/Dose (mg/kg bw)</th>
<th>3 days lesion score</th>
<th>Percentage (%) of inhibition</th>
<th>7 days lesion score</th>
<th>Percentage (%) of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>Aspirin induced ulcer (Control)</td>
<td>16.80 ± 0.11</td>
<td>0</td>
<td>16.80 ± 0.11</td>
<td>0</td>
</tr>
<tr>
<td>Group B</td>
<td>Aspirin + pre-treated with DAGP (250 mg/kg)</td>
<td>12.25** ± 0.32</td>
<td>27.08</td>
<td>8.65** ± 0.17</td>
<td>48.51</td>
</tr>
<tr>
<td>Group C</td>
<td>Aspirin + pre-treated with DAGP (500 mg/kg)</td>
<td>6.96** ± 0.91</td>
<td>58.57</td>
<td>3.25** ± 1.05</td>
<td>80.65</td>
</tr>
<tr>
<td>Group D</td>
<td>Aspirin + pre-treated with DAGP (1000 mg/kg)</td>
<td>5.88** ± 1.21</td>
<td>65.00</td>
<td>2.58** ± 0.56</td>
<td>84.64</td>
</tr>
</tbody>
</table>

Results are Mean ± SEM, for 6 replicates, *P* values: ** means significant at *P* < 0.05 when Group A (aspirin induced) vs. Group C–D (DAGP pre-treated + aspirin for 3 or 7 days) are compared.

### Table 2. Effects of *Dialium guineense* pulp (DAGP) on gastric lesions, protein levels, lipid peroxidation and antioxidant enzymes in gastric mucosa of albino Wistar rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups / Treatment (Dose)</th>
<th>Group I / Normal control</th>
<th>Group II / Albino Wistar rats received 400 mg/kg bw of aspirin suspended in 0.5% carboxy methyl cellulose via oral administration (Negative control)</th>
<th>Group III / Albino Wistar rats received 400 mg/kg bw of aspirin + pre-treatment with 1000 mg/kg bw per day of DAGP administered orally for 7 days</th>
<th>Group IV / Albino Wistar rats received 1000 mg/kg bw per day of DAGP administered orally for 7 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of lesions</td>
<td></td>
<td>0</td>
<td>16.80*** ± 0.11</td>
<td>2.58*** ± 0.56</td>
<td>0</td>
</tr>
<tr>
<td>Protein (mg/100 mg tissue)</td>
<td></td>
<td>17.92 ± 0.75b</td>
<td>10.34*** ± 0.19a</td>
<td>16.65*** ± 0.41</td>
<td>18.67 ± 0.17c</td>
</tr>
<tr>
<td>Lipid peroxide (nmol of MDA/mg protein)</td>
<td></td>
<td>5.04 ± 0.67a</td>
<td>11.83*** ± 0.93b</td>
<td>4.96*** ± 0.26</td>
<td>4.97 ± 0.29a</td>
</tr>
<tr>
<td>Superoxide dismutase (units/ mg protein)</td>
<td></td>
<td>5.92 ± 0.19b</td>
<td>3.65*** ± 1.04a</td>
<td>5.84*** ± 0.39</td>
<td>6.68 ± 0.42c</td>
</tr>
<tr>
<td>Catalase (nm of H$_2$O$_2$ decomposed/min-mg protein)</td>
<td></td>
<td>7.38 ± 0.81b</td>
<td>4.57*** ± 0.72a</td>
<td>6.92*** ± 0.33</td>
<td>7.26 ± 0.51b</td>
</tr>
<tr>
<td>Glutathione peroxidase (μg GSH utilised/min-mg protein)</td>
<td></td>
<td>162.18 ± 0.62b</td>
<td>78.44*** ± 0.23a</td>
<td>159.31*** ± 0.45</td>
<td>166.50 ±0.76c</td>
</tr>
<tr>
<td>Reduced Glutathione sulphhydril group (nm/g protein)</td>
<td></td>
<td>5.37 ± 1.02b</td>
<td>3.39*** ± 0.55a</td>
<td>4.86*** ± 0.83</td>
<td>5.40 ± 0.74b</td>
</tr>
<tr>
<td>Gluthathione-S-transferase (nmol of CDNB conjugated/ min-mg protein)</td>
<td></td>
<td>5.44 ± 0.47b</td>
<td>3.21*** ± 0.61a</td>
<td>4.85*** ± 0.28</td>
<td>5.72 ± 0.50c</td>
</tr>
</tbody>
</table>

Values are Mean ± SEM of 6 replicates in each group. Group II and Group IV was statistically compared with Group I and different letters denote significant difference at $P < 0.05$. Group III was compared with Group II and *** represent significant difference between the groups at $P < 0.05$. 
Discussion

Peptic ulcer is a clinical form of gastrointestinal disorder with many etiologies in which several causative factors play a role. Peptic ulcer has been reported to be elicited by a cellular imbalance in aggressive factors that expose the upper gastrointestinal tract to an excessive amount of acid and disruption of the mucosal integrity through compromised endogenous defense mechanism (Verma, 2010; Vijayakumar et al., 2011). Also, among the different mechanisms associated with the induction of ulcers in experimental animal models, Umamaheswari et al. (2007) reported the mechanistic roles and involvement of oxygen derived free radicals as causative agents in acute and chronic stomach ulceration. Restoration of the imbalance caused by peptic ulcers has been reasonably achieved through the use of therapeutics such as synthetic drugs and ethnomedical products including identified medicinal plants extracts with high level of antioxidants (Ngobidi et al., 2016; Rajeswari et al., 2013; Sindi and Basaprain, 2016). Proposed mechanisms of these therapeutic agents include (among others) inhibiting the secretion of gastric acid, increasing the production of mucus for defense mechanism, stabilization of the surface epithelial cell, and prevention of other causative mechanisms (Garrow and Delege, 2010). Also, reports have shown that antioxidants not only strengthen the gastric walls but protect it and other tissues from damage arising from oxidative stress (Pandiyani et al., 2009). Reports have shown that the treatment of ulcer with synthetic drugs present severe adverse clinical reactions, which include, but are not limited to, anaphylaxis reactions, hematopoietic changes, and acute interstitial nephritis including cellular and organ dysfunction (Chatterjee and Bandyopadhyay, 2014). Thus, treatment options with little or no adverse clinical reactions are preferable. Plants parts and plants products/extracts with little or no side effects have been used in practices for the treatment of diseases for decades because they contain so many bioactive compounds with inherent potential for curing human diseases (Umashankar and Shutt, 2011).

Aspirin, a potent nonsteroidal anti-inflammatory drug (NSAID) can induce the production and release of reactive oxygen species in animal models, and reports have shown that these reactive oxygen species contribute to the underlying mechanism leading to acute aspirin-induced gastric mucosal lesions (McAlindon et al., 1996; McCord, 2000). Reports have shown that agents that remove or scavenge reactive oxygen species drastically reduce gastric mucosal injury, especially those caused by reactive oxygen species (Goel et al., 2001; Robischon and Shutti, 2011). Thus, treatment options with little or no adverse clinical reactions are preferable. Plants parts and plants products/extracts with little or no side effects have been used in practices for the treatment of diseases for decades because they contain so many bioactive compounds with inherent potential for curing human diseases (Umashankar and Shutt, 2011).

The significant reduction (P < 0.05) in the protein concentration of the stomach of aspirin pre-treated albino Wistar rats (Table 2) might be attributed to the effects of toxic free radicals accumulated in the gastric mucosa cells (Jainu and Devi, 2004). This study indicated a significant increase (P < 0.05) in the levels of protein in the stomach of albino Wistar rats pre-treated with Dialium guineense pulp extract (Table 2). The implication of this finding showed that Dialium guineense pulp extract increased the protein content of the gastric mucosal tissue and thus protected it from the toxic effects of aspirin induction in the albino Wistar rats. Laine et al. (2008) and Rajeswari et al. (2013) reported similar findings for other plant extracts with high therapeutic potential for the protection of gastric mucosa. The lipid peroxidase increased significantly (P < 0.05) by aspirin induction and was reduced significantly (P < 0.05) by administering DAGP extract orally (Table 2). This implies that the ability of Dialium guineense pulp extract to scavenge reactive oxygen species provides gastrointestinal protection to aspirin-induced ulceration through its reductive effects on lipid peroxidation in the albino Wistar rats subjected to oxidative stress. The blocking of lipid peroxidation in the rats is the possible mechanism which the bioactive compounds in the Dialium guineense pulp extract exhibited; the results (Table 2) have shown that the extract exhibited significant hydroxyl radical scavenging potential, a possible mechanism of cytoprotection. Similar reports showing scavenging activity that reduces lipid peroxidation exists for S. nigrum berries (Prashanth Kumar et al., 2001), Polygonum minus aqueous leaf extract (Wasman et al., 2010) and Parkia speciosa ethanolic leaf extract (Al Batran et al., 2013).

In oxygen handling cells with increased oxidative stress, production of CAT, SOD, GST, GPX, GSH, and other antioxidants enzymes is a proactive cellular defense against cell damage due to oxidative stress. The general mechanism involves decomposing reactive oxygen species (ROS) and hydrogen peroxide (H₂O₂) produced in different aerobic metabolism by the antioxidant enzymes before the reactive oxygen species interact to form more reactive species that can cause cellular damage (Sindi and Basaprain, 2016). In this study, the increase in SOD and CAT levels were significant (Table 2). Reports have shown that SOD and CAT enzymes reduce oxidative damage caused by aspirin in the gastric mucosa in a highly specific catalytic activity (Halliwell and Gutteridge, 1985). The increased activities of SOD and CAT contributed to the effectiveness of the antioxidants activities. Thus, the defensive factors involved in the ulcer protective effects of Dialium guineense pulp extract could be ascribed to the antioxidant activities of their bioactive compounds. This observation is similar to that observed with Parkia speciosa ethanolic leaf extract (Al Batran et al., 2013).

Methanol extract of Dialium guineense pulp increased the activities of GSH in a dose-dependent manner but indicated a significant relationship (P < 0.05) between the severity of ulcer indices and the GSH levels in gastric mucosa (Table 2). La Casa (2000) reported similar observations which differ from the reports of Mutoh et al. (1991) who reported in vitro protection of gastric mucosal cells in suckling rats against gastric acid with reduced GSH. This report showed that increased GSH offered mucosal protection via its antioxidant properties (Repetto and Llesuy, 2002). In cells, GSH-Px and GST are essential for striking a balance in the level of reduced glutathione and oxidized glutathione (Vaanannenn et al., 1991). The increased levels of GSH-Px and GST activities by the methanol extract of Dialium guineense pulp showed that the plant extract contains a high level of antioxidant enzymes (Kamel et al., 2014). This is supported by the ability of DAGP extract to enhance reduced GSH levels and also to reduce lipid peroxidation (Al Batran et al., 2013; Wasman et al., 2010). In this study, the observed effects of Dialium guineense pulp extract demonstrated the protective role of its antioxidants’ constituents against mucosal injury, as well as its inhibitory effects on gastric ulcer progression (La Casa et al., 2000; Singh et al., 2002). Thus, methanol extracts...
of *Dialium guineense* pulp (DAGP) offers cytoprotection on aspirin induced ulcer in albino Wistar rats via the activities of its biologically potent active constituents with antioxidative properties. The limitation in this research was our inability to isolate, characterize and identify the actual bioactive constituent in *Dialium guineense* pulp that exhibits this anti-ulcerogenic effect due to a paucity of research funds.

## Conclusions

These study findings revealed that *Dialium guineense* pulp (DAGP) extract exhibited cytoprotective effects and reduced oxidative damage in aspirin induced gastric ulcer through its free radical scavenging potentials. The study also revealed that DAGP extract reduces lipid peroxidation and thus ultimately reduces loss of cellular functions. This study provided baseline data that supports the use of DAGP in ethnomedicine for the management of ulcer. There is a need for additional research that will fully characterize the bioactive compounds in *Dialium guineense* extract. This will ensure that the potentials of *Dialium guineense* pulp will be harnessed to the maximum for the treatment of ulcer and other oxidative stressed causative ailments in clinical subjects since this study provided evidence of DAGP antiulcer mechanism and cytoprotective effects.

## Conflict of interests

The authors declare they have no conflict of interests.

## Role of the funding source

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## References


**Supplementary materials**

**Table S1.** Composition of rat diet (Grower Pellet of Chikun Feeds, Kaduna)

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>Compositions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metabolizable Energy (ME)</td>
<td>2800 kcal/kg (min)</td>
</tr>
<tr>
<td>Crude Protein (CP)</td>
<td>18.00% (min)</td>
</tr>
<tr>
<td>Crude Fat</td>
<td>5.00% (max)</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>54.4% (min)</td>
</tr>
<tr>
<td>Crude Fibre (CF)</td>
<td>8.00% (max)</td>
</tr>
<tr>
<td>Calcium</td>
<td>1.00% (min)</td>
</tr>
<tr>
<td>Phosphorus, available</td>
<td>0.40% (min)</td>
</tr>
<tr>
<td>Lysine</td>
<td>1.00% (min)</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.45% (min)</td>
</tr>
<tr>
<td>Methionine + Cystine</td>
<td>0.80% (min)</td>
</tr>
<tr>
<td>Threonine</td>
<td>0.60% (min)</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>0.20% (min)</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>44 IU/g</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>80 IU/kg</td>
</tr>
<tr>
<td>Vitamin K (menadione)</td>
<td>3.4 mg/kg</td>
</tr>
<tr>
<td>Vitamin B₁</td>
<td>92 mg/kg</td>
</tr>
<tr>
<td>Vitamin B₂</td>
<td>8.0 mg/kg</td>
</tr>
<tr>
<td>Niacin</td>
<td>60 mg/kg</td>
</tr>
<tr>
<td>Vitamin B₆</td>
<td>12 mg/kg</td>
</tr>
<tr>
<td>Pantothenic acid</td>
<td>24 mg/kg</td>
</tr>
<tr>
<td>Biotin</td>
<td>0.28 mg/kg</td>
</tr>
<tr>
<td>Folate</td>
<td>4.2 mg/kg</td>
</tr>
<tr>
<td>Vitamin D₃</td>
<td>1.5 IU/g</td>
</tr>
<tr>
<td>Vitamin B₁₂</td>
<td>0.019 mg/kg</td>
</tr>
</tbody>
</table>

Composition information is on the food bag labels (as at September 26, 2018).
Table S2. Body weight (g) and daily food intake (g) of control and treated albino Wistar rats over the experimental treatment period

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Treatment/Dose (mg/kg bw)</th>
<th>Initial body weight</th>
<th>Final body weight</th>
<th>Body weight change</th>
<th>Food intake</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Period of treatment: 3 days</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group A</td>
<td>Normal control (rat chow only)</td>
<td>162.55 ± 1.05</td>
<td>163.83 ± 1.33</td>
<td>1.23 ± 0.59</td>
<td>18.78 ± 0.17</td>
</tr>
<tr>
<td>Group B&lt;sub&gt;1&lt;/sub&gt;</td>
<td>Treatment with 250 mg/kg bw per day of DAGP administered orally</td>
<td>158.98 ± 1.17</td>
<td>160.28 ± 1.06</td>
<td>1.30 ± 0.75</td>
<td>18.94 ± 0.25</td>
</tr>
<tr>
<td>Group C&lt;sub&gt;1&lt;/sub&gt;</td>
<td>Treatment with 500 mg/kg bw per day of DAGP administered orally</td>
<td>161.50 ± 1.50</td>
<td>163.45 ± 1.35</td>
<td>1.95 ± 0.82&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.12 ± 0.13&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group D&lt;sub&gt;1&lt;/sub&gt;</td>
<td>Treatment with 1000 mg/kg bw per day of DAGP administered orally</td>
<td>164.25 ± 2.11</td>
<td>166.29 ± 1.15</td>
<td>2.04 ± 0.65&lt;sup&gt;b&lt;/sup&gt;</td>
<td>19.27 ± 0.29&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Period of treatment: 7 days</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group A</td>
<td>Normal control (rat chow only)</td>
<td>161.42 ± 1.31</td>
<td>163.09 ± 1.41</td>
<td>1.67 ± 1.01</td>
<td>18.81 ± 0.17</td>
</tr>
<tr>
<td>Group B&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Treatment with 250 mg/kg bw per day of DAGP administered orally</td>
<td>166.30 ± 2.04</td>
<td>168.11 ± 2.13</td>
<td>1.81 ± 0.85&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.30 ± 0.15&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group C&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Treatment with 500 mg/kg bw per day of DAGP administered orally</td>
<td>162.08 ± 1.18</td>
<td>164.89 ± 2.03</td>
<td>2.81 ± 0.74&lt;sup&gt;b&lt;/sup&gt;</td>
<td>19.32 ± 0.18&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group D&lt;sub&gt;3&lt;/sub&gt;</td>
<td>Treatment with 1000 mg/kg bw per day of DAGP administered orally</td>
<td>165.50 ± 1.21</td>
<td>168.97 ± 1.85</td>
<td>3.47 ± 0.93&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>19.53 ± 0.21&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± Standard error of mean (SEM), n - 6; * significantly different from control at P < 0.05; Mean values with different alphabet along the same column are significantly different at P < 0.05.

Food intake (g) and body weight changes (g) were measured as follows:

Daily food intake = food supplied to each rat – (leftover + spilled food)

Body weight change = final weight – initial weight of each rat