J Appl Biomed
DOI: 10.32725/jab.2021.015
journal homepage: http://jab.zsf.jcu.cz

Journal of Applied Biomedicine

Original research article

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

Nwogo Ajuka Obasi [1]*, Chinyere Aloke [1], Stella Eberechukwu Obasi [2], Chinedu Ogbonnia Egwu [1, 3], Ademola Clement Famurewa [1], Patience Nkemjika Ogbu [1]

[1] *Alex Ekwueme Federal University Ndufu-Alike, Department of Medical Biochemistry, Ikwo, Nigeria*
[2] *Akanu Ibiam Federal Polytechnic Unwana, Department of Science Laboratory Technology, Unwana, Nigeria*
[3] *Paul Sabatier University Toulouse III, Department of Biochemistry, Toulouse, France*

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 $P \leq 0.05$. The results showed that *Dialium guineense* pulp caused a significant decrease ($P \leq 0.05$) 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 ($P \leq 0.05$) in lipid peroxidation and a decrease ($P \leq 0.05$) 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 (Søberg et al., 2010). These factors can expose the upper gastro-intestinal tract to an excessive amount of acid (Abdulla 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

* **Corresponding author:** Nwogo Ajuka Obasi, Alex Ekwueme Federal University Ndufu-Alike, Department of Medical Biochemistry, Ikwo, Nigeria; e-mail: naobasi@yahoo.com; ajuka.obasi@funai.edu.ng
http://doi.org/10.32725/jab.2021.015
Submitted: 2020-01-22 • Accepted: 2021-06-07 • Prepublished online: 2021-06-10
J Appl Biomed 19/3: 170–178 • EISSN 1214-0287 • ISSN 1214-021X

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).

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 *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 *Dialium guineese* pulp (DAGP) on aspirin-induced damage in gastric mucosa through its effect on the antioxidative enzymes.

Materials and methods

Collection and identification of plant materials

The ripe fruits (Fig. 1) were collected from the *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.

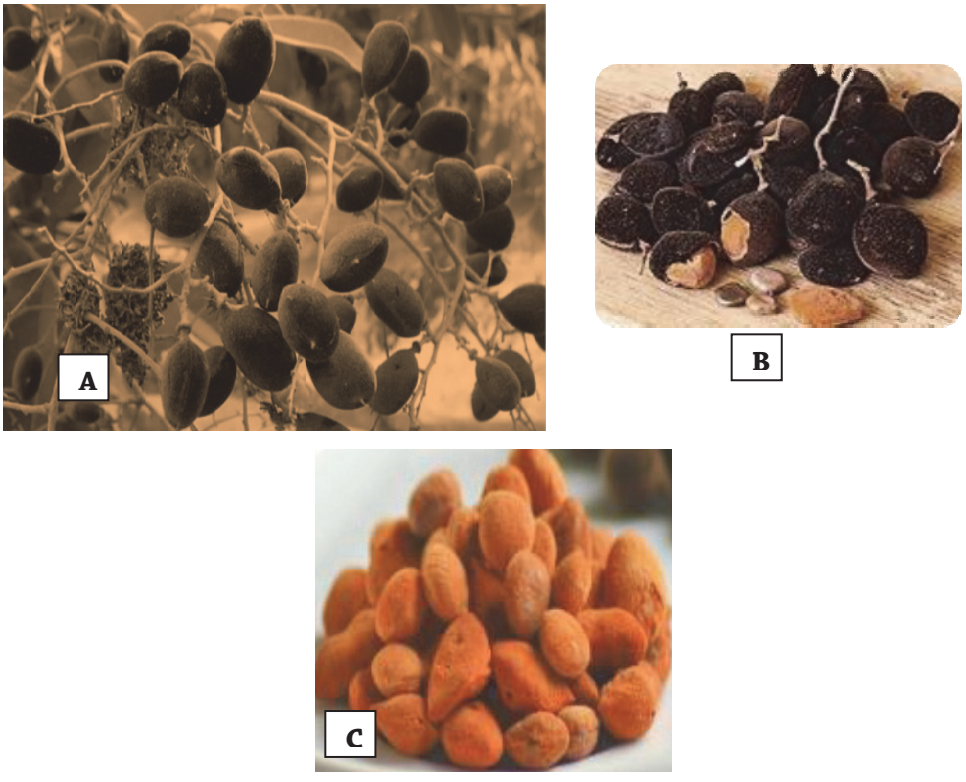

Fig. 1. *Dialium guineense* fruits (**A**) Unshelled *Dialium guineense* fruits with the stalk on the tree, (**B**) Harvested unshelled *Dialium guineense* fruits with the seed coat and (**C**) Shelled *Dialium guineense* fruits with seed coats removed exposing the edible portion used for the study.

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 cryodesiccated 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 Unwana 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 (LD_{50}) 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 administered 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 (LD_{50}) 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 suspended in 0.5% carboxy methyl cellulose via oral administration (control).

Group B – 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 B_2 – Albino Wistar rats received 400 mg/kg bw of aspirin + pre-treatment with 250 mg/kg bw per day of DAGP administered orally for 7 days.

Group C – Albino Wistar rats received 400 mg/kg bw of aspirin + pre-treatment with 500 mg/kg bw per day of DAGP administered orally for 3 days.

Group C_2 – 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 3 days.

Group D_2 – 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)

$$\textit{Curative ratio (CR)} = (LC - LT/LC)\ 100 \quad (i)$$

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 with1000 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 scrapping the gastric mucosa using a scrapper 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

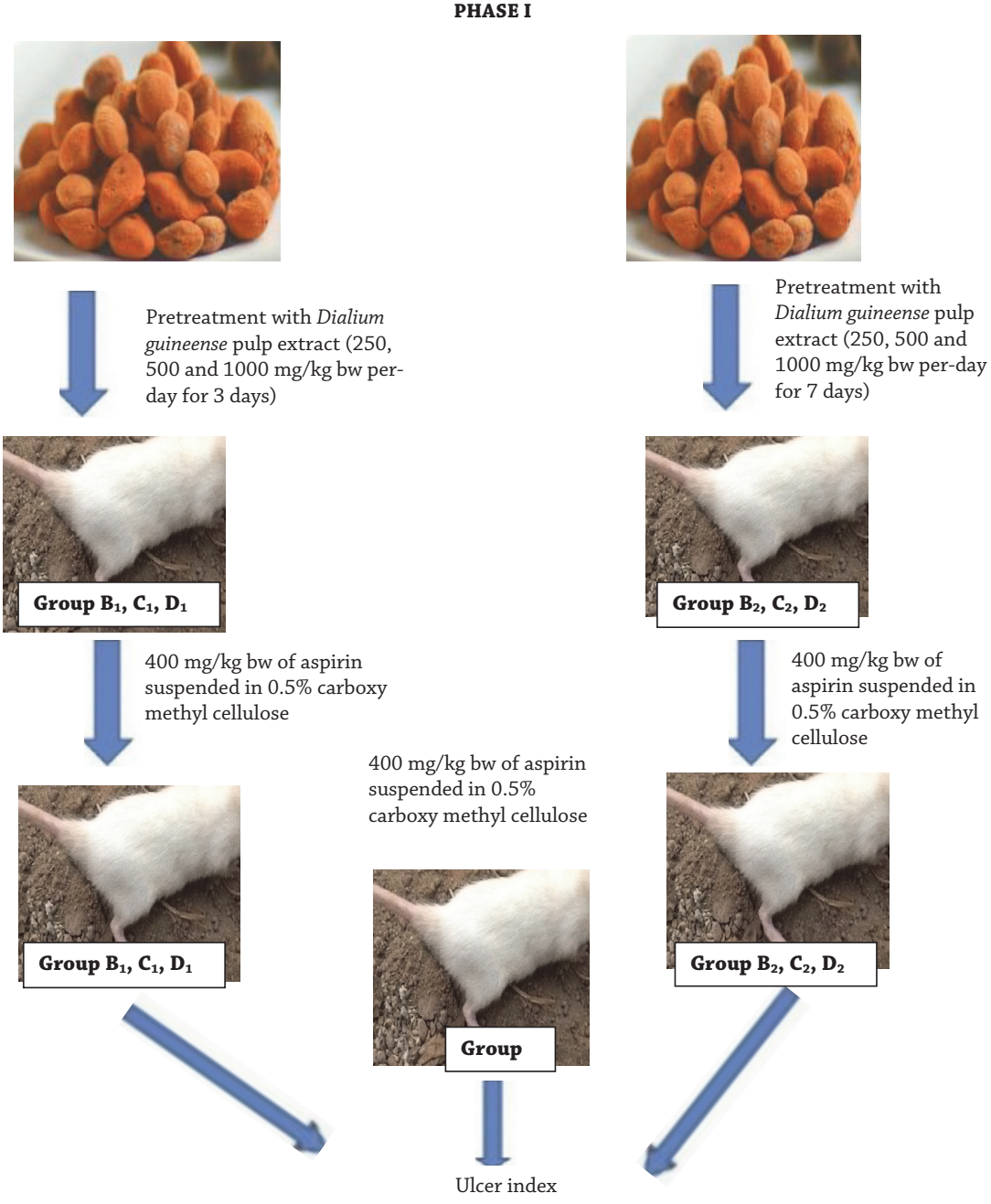

Fig. 2. Overview of experimental design Phase I

Sizer (1952) via the decomposition of H_2O_2. The Rotruck et al. (1973) method was used to measure the glutathione peroxidase (GPX), using H_2O_2 as substrate, while the method outlined by Habig et al. (1974) which used 1-chloro-2,4-dinitrobenzene as substrate was employed to estimate the activities of glutathione-S-transferase (GST).

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 $P < 0.05$.

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.

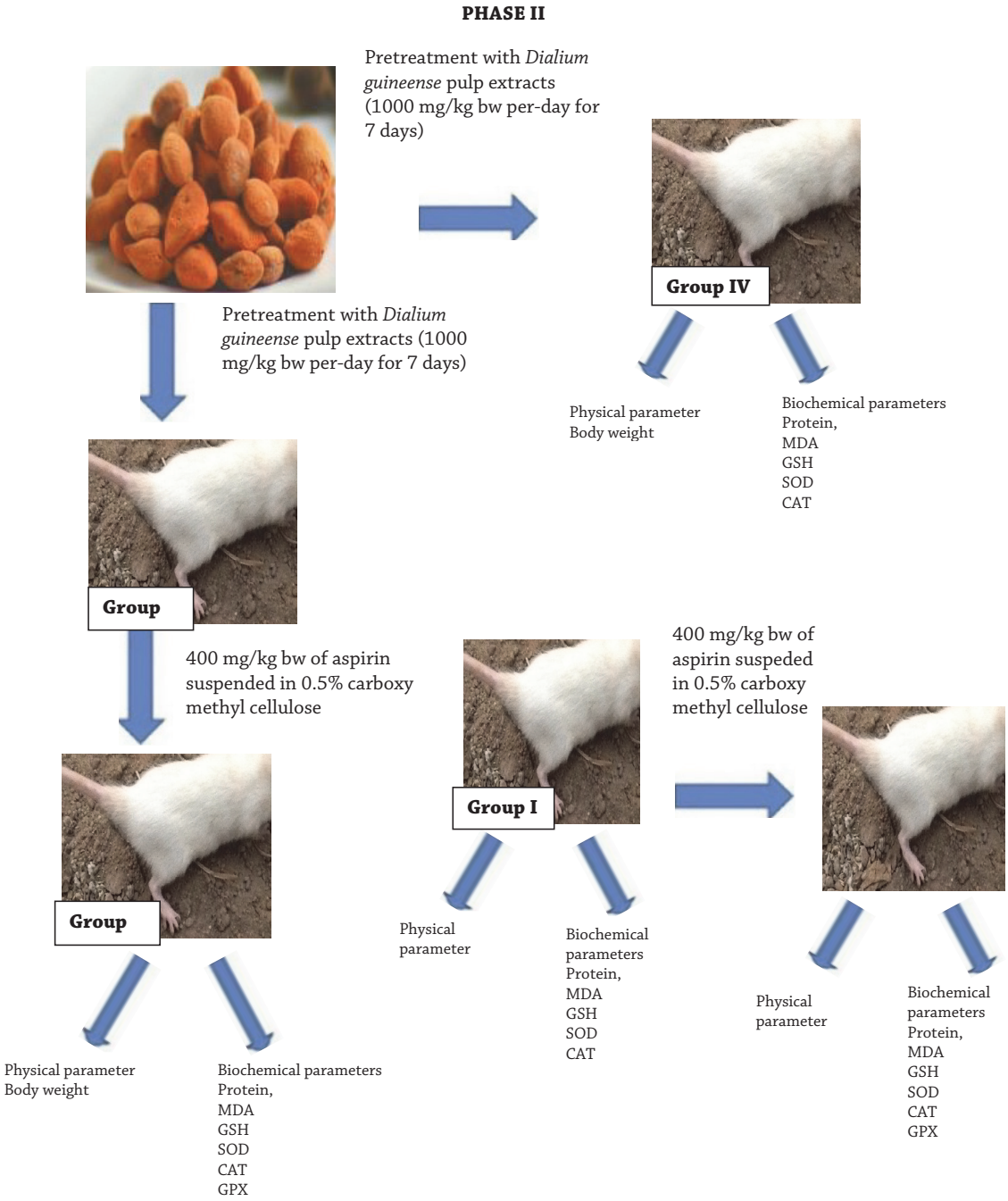

Fig. 3. Overview of experimental design Phase II

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

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

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.

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

Parameters	Groups / Treatment (Dose)			
	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
Number of lesions	0	16.80*** ± 0.11	2.58*** ± 0.56	0
Protein (mg/100 mg tissue)	17.92 ± 0.75^{b}	10.34*** ± 0.19^{a}	16.65*** ± 0.41	18.67 ± 0.17^{c}
Lipid peroxide (nmoles of MDA/mg protein)	5.04 ± 0.67^{a}	11.83*** ± 0.93^{b}	4.98*** ± 0.26	4.97 ± 0.29^{a}
Superoxide dismutase (units/ mg protein)	5.92 ± 0.19^{b}	3.65*** ± 1.04^{a}	5.84*** ± 0.39	6.68 ± 0.42^{c}
Catalase activity (nm of H_2O_2 decomposed/min-mg protein)	7.38 ± 0.81^{b}	4.57*** ± 0.72^{a}	6.92*** ± 0.33	7.26 ± 0.51^{b}
Glutathione peroxidase (μg GSH utilised/min-mg protein)	162.18 ± 0.62^{b}	78.44*** ± 0.23^{a}	159.31*** ± 0.45	166.50 ±0.76^{c}
Reduced Gluthione sulphydryl group (nm/g protein)	5.37 ± 1.02^{b}	3.39*** ± 0.55^{a}	4.86*** ± 0.83	5.40 ± 0.74^{b}
Glutathione-S-transferase (nmoles of CDNB conjugated/ min-mg protein)	5.44 ± 0.47^{b}	3.21*** ± 0.61^{a}	4.85*** ± 0.28	5.72 ± 0.50^{c}

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 gastro-intestinal 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 ethnomedicinal 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 (Pandiyan 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 Shutti, 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; Verma et al., 2012). This study showed that methanol extract of *Dialium guineense* pulp significantly ($P < 0.05$) decreased aspirin-induced ulcers in albino Wistar rats by inhibiting the increase in gastric mucosa lesion areas (Table 1). This result compares favorably with the inhibitive effects of *Polygonum minus* aqueous leaf extract (Wasman et al., 2010), *Solanum nigrum* berries (Jainu and Devi, 2004), *Parkia speciosa* ethanolic leaf extract (Al Batran et al., 2013), *Musa paradisiacal* Linn peel (Ezekwesili et al., 2014) and *Dialium guineense* leaf extract (Balogun et al., 2013) on mucosal lesions of albino Wistar rats.

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 antioxidant 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 (H_2O_2) 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 (Vaananenn 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 perioxidation (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 that they have no conflict of interests.

Role of the funding source

There was no external funding received for this research.

Acknowledgements

The authors are grateful to the Chief Executive/Rector, Akanu Ibiam Federal Polytechnic Unwana, Nigeria, Venerable (Dr) Ogbonnia Ibe-Enwo for granting the authors free access to laboratory facilities in the Department of Science Laboratory Technology of his institution for the execution of this work, and to all the Technological staff of the Department of Science Laboratory Technology for their cooperation and technical assistance.

References

Abdulla MA, AL-Bayaty FH, Younis LT, Abu-Hassan MI (2010). Anti-ulcer activity of *Centella asiatica* leaf extract against ethanol-induced gastric mucosal injury in rats. J Med Plants Res 4(13): 1253–1259. DOI: 10.5897/JMPR10.197.

Adeleye AO, Ajiboye TO, Iliasu GA, Abdussalam FA, Balogun A, Ojewuyi OB, Yakubu MT (2014). Phenolic extract of *Dialium guineense* pulp enhances reactive oxygen species detoxification in aflatoxin B_1 hepatocarcinogenesis. J Med Food 17(8): 875–885. DOI: 10.1089/jmf.2013.0157.

Adumanya OCU, Osuji CN, Obi-Adumanya GA, Akunna TO (2013). Antiplasmodial effect of some medicinal plants (*Picralima nitida* and *Dialium guineense*) and their combination with artesunate. Int J A PS BMS 2(4): 189–194.

Agrawal AK, Rao CV, Sariam K, Joshi VK, Goel RK (2000). Effect of *Piper longum* L., *Zingiber officinalis* L. and *Ferula* species on gastric ulceration and secretion in rats. Indian J Exp Biol 38(10): 994–998.

Al Batran R, Al-Bayaty F, Jamil Al-Obaidi MM, Abdualkader AM, Hadi HA, Ali HM, Abdulla MA (2013). *In vivo* antioxidant and antiulcer activity of *Parkia speciosa* ethanolic leaf extract against ethanol-induced gastric ulcer in rats. PLoS ONE 8(5): e64751. DOI: 10.1371/journal.pone.0064751.

Atawodi SE (2005). Comparative *in vitro* trypanocidal activities of petroleum ether, chloroform, methanol, and aqueous extracts of some Nigerian savannah plants. Afr J Biotechnol 4(2): 177–182.

Balogun ME, Oji JO, Besong EE, Umahi GO (2013). Evaluation of the anti-ulcer properties of aqueous leaf extract of *Dialium guineense* (Velvet tamarind) on experimentally induced ulcer models in rats. Intl J Dev Res 3: 106–110.

Bardi D, Khan MAS, Sabri S, Kadir F, Mahmood A, Amin ZA, et al. (2011). Antiulcerogenic activity of *Typhonium flagelliforme* aqueous leaf extract against ethanol-induced gastric mucosal injury in rats. Sci Res Essays 6(15): 3232–3239. DOI: 10.5897/SRE11.335.

Beers RF, Sizer IW (1952). A spectrophotometric method for measuring the breakdown of H_2O_2 by catalase. J Biol Chem 195(1): 133–140.

Besong EE, Balogun ME, Djobissie SFA, Obu DC, Obimma JN (2016). Medicinal and economic value of *Dialium guineense*. Afr J Biomed Res 19(3): 163–170.

Chatterjee A, Bandyopadhyay SK (2014). Herbal Remedy: An Alternate Therapy of Nonsteroidal Anti-Inflammatory Drug Induced Gastric Ulcer Healing. Ulcers 2014(1–4). DOI: 10.1155/2014/361586.

Das UN, Vijay Kumar K, Krishna Mohan I (1994). Lipid Peroxides and Essential Fatty Acids in Patients with Diabetes Mellitus and Diabetic Nephropathy. J Nutr Med 4(2): 149–155. DOI: 10.3109/13590849409034550.

Dressler S, Schmidt M, Zizka G (2014). African Plants – A Photo Guide. Entry: *"Dialium guineense"*. [online] [2019-10-13]. Available from: www.africanplants.senckenberg.de Frankfurt/Main: Forschungsinstitut Senckenberg

Dursun H, Bilici M, Albayrak F, Ozturk C, Saglam MB, Alp HH, et al. (2009). Antiulcer activity of fluvoxamine in rats and its effect on oxidant and antioxidant parameters in stomach tissue. BMC Gastroenterol 9: 36. DOI: 10.1186/1471-230X-9-36.

Ellman GL (1959). Tissus sulphydryl groups. Arch Biochem Biophys 82(1): 70–77. DOI: 10.1016/0003-9861(59)90090-6.

Ezeja MI, Omeh YS, Ezeigbo I, Ekechukwu A (2011). Evaluation of the analgesic activity of the methanolic stem bark extract of *Dialium guineense* (wild). Ann Med Health Sci Res 1(1): 55–62.

Ezekwesili CN, Ghasi S, Adindu CS, Mefoh NC (2014). Evalution of the anti-ulcer property of aqueous extract of unripe *Musa paradisiacal* Linn. peel in Wistar rats. Afr J Pharm Pharmacol 8(39): 1006–1011. DOI: 10.5897/AJPP2014.4007.

Garrow D, Delegge MH (2010). Risk factor for gastrointestinal ulcer disease in the US population. Dig Dis Sci 55(1): 66–72. DOI: 10.1007/s10620-008-0708-x.

Goel RK, Sairam K, Rao CV (2001). Role of gastric antioxidant and anti-*Helicobactor pylori* activities in antiulcerogenic activity of plantain banana (*Musa sapientum* var. *paradisiaca*). Indian J Exp Biol 39(7): 719–722.

Habig WH, Pabst MJ, Jakoby WB (1974). Glutathione S-transferase. The first enzymatic step in mercapturic acid formation. J Biol Chem 249(22): 7130–7139.

Halliwell B, Gutteridge JM (1985). The importance of free radicals and catalytic metal ions in human diseases. Mol Aspects Med 8(2): 89–193. DOI: 10.1016/0098-2997(85)90001-9.

Itatsu T, Nagahara A, Hojo M, Miyazaki A, Murai T, Nakajima M, et al. (2011). Use of selective serotonin reuptake inhibitors and upper gastrointestinal disease. Intern Med 50(7): 713–717. DOI: 10.2169/internalmedicine.50.4644.

Jaarin K, Gapor MT, Nafeeza MI, Fauzee AM (2002). Effect of various doses of palm vitamin E and tocopherol on aspirin-induced gastric lesions in rats. Int J Exp Path 83: 295–301. DOI: 10.1046/j.1365-2613.2002.00242.x.

Jainu M, Devi CSS (2004). Antioxidant Effect of Methanolic Extract of *Solanum nigrum* Berries on Aspirin Induced Gastric Mucosal Injury. Indian J Clin Biochem 19(1): 57–61. DOI: 10.1007/BF02872391.

Kamel MA, Hamza RZ, Abdel-Hamid NE, Mahmoud FA (2014). Anti-ulcer and gastro protective effects of fenugreek, ginger and peppermint oils in experimentally induced gastric ulcer in rats. J Chem Pharm Res 6(2): 451–468.

Konturek PC, Brzozowski T, Konturek SJ, Pajdo R, Konturek JE, Kwiecien S, et al. (1999). Apoptosis in gastric mucosa with stress-induced gastric ulcers. J Physiol Pharmacol 50(2): 211–225.

La Casa C, Villegas I, Alarcon de La Lastra C, Motilva V, Martín Calero MJ (2000). Evidence for protective and antioxidant properties of rutin, a natural flavone, against ethanol induced gastric lesions. J Ethnopharmacol 71(1–2): 45–53. DOI: 10.1016/s0378-8741(99)00174-9.

Laine L, Takeuchi K, Tarnawski A (2008). Gastric mucosal defense and cytoprotection: bench to bedside. Gastroenterology 135(1): 41–60. DOI: 10.1053/j.gastro.2008.05.030.

Lanas A (2009). Nonsteroidal antiinflammatory drugs and cyclooxygenase inhibition in the gastrointestinal tract: a trip from peptic ulcer to colon cancer. Am J Med Sci 338(2): 96–106. DOI: 10.1097/maj.0b013e3181ad8cd3.

Lorke D (1983). A New Approach to Acute Toxicity Testing. Arch Toxicol 54(4): 275–287. DOI: 10.1007/BF01234480.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951). Protein measurement with Folin phenol reagent. J Biol Chem 193(1): 265–278.

Mahmood AA, Al-Bayaty FH, Salmah I, Nor Syuhada AB, Harita H, Mughrabi FF (2011). Enhancement of gastric ulcer by *Areca catechu* nut in ethanol-induced gastric mucosal injuries in rats. J Med Plant Res 5(12): 2562–2569.

McAlindon ME, Muller AF, Filipowicz B, Hawkey CJ (1996). Effect of allopurinol, sulphasalazine and vitamin C on aspirin induced gastroduodenal injury in human volunteers. Gut 38(4): 518–524. DOI: 10.1136/gut.38.4.518.

McCord JM (2000). The evolution of free radicals and oxidative stress. Am J Med 108(8): 652–659. DOI: 10.1016/s0002-9343(00)00412-5.

Misra HP, Fridovich I (1972). The role of superoxide anion in the autoxidation of epinephrine and a simple assay of superoxide dismutase. J Biol Chem 247(10): 3170–3175.

Mutoh H, Ota S, Hirashi H, Ivey KJ, Terano A, Sugimoto T (1991). Reduced glutathione protects cultured gastric mucosal cells from suckling rats against acid. Am J Physiol 261(1 Pt. 1): G65–70. DOI: 10.1152/ajpgi.1991.261.1.G65.

Ngobidi KC, Igbokwe GE, Ajayi AA, Otuchristian G, Obasi SE, Osigwe AO, Adindu SC (2016). Anti-Ulcerative Effect of Ethanol Leaf Extract of *Camellia sinensis* on Albino Rats Induced Ulcer with Ethanol. Int J Res Pharm Biosci 3(7): 28–33.

Obasi NA, Kalu KM, Okorie UC, Otuchristian G (2013). Hypoglycemic effects of Aqueous and Methanolic Leaf Extracts of *Vitex doniana* on Alloxan Induced Diabetic Albino Rats. J Med Sci 13(8): 700–707. DOI: 10.3923/jms.2013.700.707.

Orji JO, Alo MN, Anyim C, Okonkwo EC (2012). Antibacterial activities of crude leaf and bark extracts of "icheku" D*ialium guineense* on bacterial isolates from bronchitis patients. J Pharm Biol Sc 1: 22–25.

Paiotti AP, Marchi P, Miszputen SJ, Oshima CT, Franco M, Ribeiro DA (2012). The role of nonsteroidal antiinflammatory drugs and cyclooxygenase-2 inhibitors on experimental colitis. In Vivo 26(3): 381–393.

Pandiyan R, Velu RK, Chandrasekaran SK, Bashiyam P (2009). Effect on extracts of *Stevia rebaudiana* Bertoni. in ethanol induced gastric ulcer by using Wistar rats. Recent Res Sci Technol 1(3): 127–130.

Prashanth Kumar V, Shashidhara S, Kumar MM, Sridhara BY (2001). Cytoprotective role of *Solanum nigrum* against gentamicin-induced kidney cell (Vero cells) damage *in vitro*. Fitoterapia 72(5): 481–486. DOI: 10.1016/s0367-326x(01)00266-0.

Rajeswari M, Gurumurthy S, Kamat S (2013). Anti gastritic and antiulcerogenic effects of *Solanum nigrum* in laboratory animals. Int J Nutr Food Sci 2(6): 266–271.

Repetto MG, Llesuy SF (2002). Antioxidant properties of natural compounds used in popular medicine for gastric ulcers. Braz J Med Biol Res 35(5): 523–534. DOI: 10.1590/s0100-879x2002000500003.

Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG (1973). Selenium: biochemical role as component of glutathione peroxidase. Science 179(4073): 588–590. DOI: 10.1126/science.179.4073.588.

Salim AS (1992). Use of scavenging oxygen-derived free radicals to protect the rat against aspirin- and ethanol-induced erosive gastritis. J Pharm Sci 81(9): 943–946. DOI: 10.1002/jps.2600810921.

Sindi HA, Basaprain R (2016). Protective Effect of Ginger and Cinnamon Aqueous Extracts against Aspirin-Induced Peptic Ulcer. World Appl Sci J 34(11): 1436–1448. DOI: 10.5829/idosi.wasj.2016.1436.1448.

Singh P, Bhargava VK, Garg SK (2002). Effect of melatonin and beta-carotene on indomethacin induced gastric mucosal injury. Indian J Physiol Pharmacol 46(2): 229–234.

Søberg T, Hofstad B, Sandvik L, Johansen M, Lygren I (2010). Risk factors for peptic ulcer bleeding. Tidsskr Nor Legeforen 130: 1135–1139. DOI: 10.4045/tidsskr.08.0693.

Sumbul S, Ahmad MA, Mohd A, Mohd A (2011). Role of phenolic compounds in peptic ulcer: An overview. J Pharm Bioallied Sci 3(3): 361–367. DOI: 10.4103/0975-7406.84437.

Umamaheswari M, Asokkumar K, Rathidevi R, Sivashanmugam AT, Subhadradevi V, Ravi TK (2007). Antiulcer and *in vitro* antioxidant activities of *Jasminum grandiflorum* L. J Ethnopharmacol 110(3): 464–470. DOI: 10.1016/j.jep.2006.10.017.

Umashankar M, Shutti S (2011). Traditional Indian Herbal Medicine Used as Antipyretic, Antiulcer, Anti-Diabetic and Anticancer: A Review. IJRPC 1(4): 1152–1159.

Vaananen PM, Meddings JB, Wallace JL (1991). Role of oxygen-derived free radicals in indomethacin-induced gastric injury. Am J Physiol 261(3 Pt 1): G470–475. DOI: 10.1152/ajpgi.1991.261.3.G470.

Verma M (2010). A Review on Peptic Ulcer: A Global Threat. J Pharm Res 3(9): 2088–2091.

Verma VK, Singh N, Saxena P, Singh R (2012). Anti-Ulcer and Antioxidant Activity of *Moringa oleifera* (Lam) Leaves against Aspirin and Ethanol Induced Gastric Ulcer in Rats. Int Res J Pharma 2(2): 46–57.

Vijayakumar M, Bavani Eswaran M, Ojha SK, Rao CV, Rawat AKS (2011). Antiulcer Activity of Hydroalchol Extract of *Momordica dioica* Roxb. Fruit. Indian J Pharm Sci 73 (5): 572–577. DOI: 10.4103/0250-474X.99018.

Wasman SQ, Mahmood AA, Salehhuddin H, Zahra AA, Salmah I (2010). Cytoprotective activities of *Polygonum minus* aqueous leaf extract on ethanol-induced gastric ulcer in rats. J Med Plants Res 4(24): 2658–2665. DOI: 10.5897/JMPR09.412.

Yeomans ND, Hawkey CJ, Brailsford W, Næsdal J (2009). Gastroduodenal toxicity of low-dose acetylsalicylic acid: a comparison with non-steroidal anti-inflammatory drugs. Curr Med Res Opin 25(11): 2785–2793. DOI: 10.1185/03007990903212682.