



Anti-ulcerogenic Activity of *Dialium guineense* Fruit Pulp Meal-based Diet in Aspirin-induced Ulcerogenic Rats

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Abstract: The study aimed at investigating the anti-ulcerogenic effects of the meal-based diet of the fruit pulp of *Dialium guineense* (Family: *Fabaceae*) at 5% - 100% inclusion levels in aspirin-induced ulcerogenic rats. Eighty eight albino rats of both sexes weighing 167.09 ± 6.78 g were assigned into two groups: A (8 rats) and B (80 rats). Animals in Group B were made ulcerogenic by administration of aspirin (200mg/kg bodyweight {bwt}) for 3 consecutive days and confirmed by determining the levels of nitric oxide and pepsinogen concentration in the serum. These animals were later re-assigned into thirteen groups as non-treated, Omeprazole (0.045 mg/kg bwt) treated and *Dialium guineense* fruit-pulp meal-based diet treated (5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and 100% inclusion levels) and they were maintained on their respective diet and drug for five weeks. The acid secretory parameters (Ulcer index, gastric pH, gastric volume and total acidity) as well as the glycoprotein concentration (hexose, fucose, hexosamine, sialic acid, total carbohydrate: protein) were determined using standard methods. The study revealed that aspirin significantly ($p < 0.05$) increased the concentration of pepsinogen, gastric volume and acidity, ulcer index whereas it decreased the gastric pH, concentrations of nitric oxide, hexose, fucose, hexosamine, sialic acid as well as the total carbohydrate: protein ratio significantly ($p < 0.05$). In contrast, the fruit pulp meal-based diet at 5% - 40% inclusion levels significantly reversed ($p > 0.05$) the levels of these parameters with the 30% and 40% inclusion levels comparing favourably ($p < 0.05$) with ulcerogenic rats treated with Omeprazole. Overall, the results suggest that the meal-based diet of *Dialium guineense* possesses anti-ulcer activity optimally at 30% and 40% inclusion levels and therefore support its usage in folklore medicine.

KEYWORDS: Aspirin, *Dialium guineense*, *Fabaceae*, Omeprazole, Ulcer index

1.0 Introduction

The endocrine, exocrine and paracrine secretions of the gastrointestinal tract (GIT) play a pivotal role in the digestion and absorption of food and drugs (Bhavani *et al.*, 2012). Disturbances in secretory and other physiological functions of the GIT as a whole can lead to several pathologies such as diarrhoea, appendicitis, cancer, ulcer *e.t.c.* (Bhavani *et al.*, 2012).

An ulcer is an eroded area of the skin or a mucous membrane, marked by tissue disintegration (Magaji *et al.*, 2007). Ulcers that occur in the lining mucosa of the gastrointestinal tract are referred to as the gastrointestinal tract ulcer and this type of ulceration can occur in the

stomach (gastric ulcer), duodenum (duodenal ulcer), oesophagus (oesophagus) or Merkel's diverticulum (Merkel's diverticulum ulcer) depending on the site of the ulceration (Magaji *et al.*, 2007). Gastrointestinal tract ulceration is the most predominant of the gastrointestinal diseases (Malfertheiner *et al.*, 2009).

The pathophysiology of this disease involves an imbalance between aggressive/offensive (acid and pepsin) and defensive factors (prostaglandins, nitric oxide, bicarbonate, mucus) (Hoogerwerf and Pasricha, 2001). In like manner, there are also exogenous factors which induce this imbalance and this include the use of non-steroidal anti-inflammatory drugs (NSAIDs), *Helicobacter pylori* infection, stress, smoking, alcohol intake (Peckenpaugh and Poleman, 1997). There are a variety of orthodox

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drugs used for the management of this disease and this include H₂ – receptors (ranitidine, famotidine), proton-pump inhibitors (omeprazole, lansoprazole), antibiotics (metronidazole, amoxicillin, clarithromycin etc), antacids and other drugs (Shimokawa *et al.*, 1996). Although, these conventional drugs which have been pharmacologically well characterized are proven to be beneficial, the adverse effects and drug-drug interactions highlight the need for better treatment modalities for gastrointestinal tract ulceration of any kind (Bhavani *et al.*, 2012). Recent studies found that different substances from plant sources not only afford gastroprotection but also accelerate ulcer healing (Magaji *et al.*, 2007). There are various plant-originated “gastroprotectors” with different composition used either as decoction, concoction or even as food additive or supplement to combat gastrointestinal ulceration in clinical and folk medicine by many countries (Magaji *et al.*, 2007). Some of these include *Diodia sarmentosa* (Rubiaceae), *Cassia nigricans* (Celastraceae), *Ficus exasperata* (Moraceae) and *Synclisia scabrida* (Menispermaceae) (Bhavani *et al.*, 2012). In Nigeria, ethnobotanical survey also revealed the use of *Dialium guineense* fruit pulp as a potent anti-ulcer agent.

Dialium guineense belongs to the family of Fabaceae. It is a woody plant that grows up to 15 m high in the rain forest region of West Africa (Okegbile and Taiwo, 1990). The seed is embedded in a dry brownish sweet sour and edible pulp (Lewis, 1978). The small, black, velvety fruits are very conspicuous and distinctive. Several medicinal properties have been attributed to the fruit pulp, leaves and seeds of the plant which include anti-diarrhoeal and anti-microbial (Odukoya *et al.*, 1996).

However, there are no scientific studies that have substantiated the acclaimed anti-ulcerogenic potential of the *Dialium guineense* fruit pulp. Therefore the present study is aimed at evaluating the anti-ulcerogenic potential of *Dialium guineense* fruit pulp meal-based diet in aspirin-induced ulcerogenic rats.

2.0 Materials and Methods

2.1 Plant Material and Authentication

The fruit pulp of *Dialium guineense* purchased from Dawanu Market in Kano City, Nigeria was authenticated in the Department of Plant Biology, University of Ilorin, Ilorin, Nigeria. A voucher specimen (UIH1064) was deposited in the Herbarium of the Department.

2.2 Laboratory Animals

Eighty eight albino rats (*Rattus norvegicus*) of both sexes (167.09 ± 6.78g, 5-7weeks old) were obtained from the Animal Holding Unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. They were kept in well-ventilated house conditions with free access to rat pellets (Premier Feed Mills Company Limited, Ibadan) and tap water before the commencement of the experiment.

2.3 Feed Ingredients

Yellow maize (*Zea mays*) seed, cellulose (corn corb) and soybean were obtained from ‘Oja Oba’ market in Ilorin, Nigeria. Soybean oil was a product of Sunola Refined Soybeans, Kewalram Nigeria Limited, Nigeria. Vitamin/Mineral mix, Lysine and D-Methionine were products of Rofat Feed Nigeria Limited, Ilorin, Nigeria.

2.4 Drugs and Chemicals

Aspirin was a product of Juhel Nigeria Ltd., Nigeria while Omeprazole was a product of Jiangsu Ruilian Pharm.Co. Ltd.China, USA. All other chemicals and reagents used for this research were of analytical grade and were obtained from Randox laboratories Co-Artrim, United Kingdom.

2.5 Preparation of *Dialium guineense* fruit pulp powder

Fresh fruit pulps of *Dialium guineense* were manually removed from the seed after expunging it from the seed coat. They were then oven-dried at 40°C and pulverized in an electric blender (Super Master Electrical Appliance Industries Co., Kyoto, Japan) and then stored for later use. For the secondary plant metabolites, a known weight (10 g) of the fruit pulp of *Dialium*

guineense was extracted in 200ml of distilled water for 48 hours at room temperature.

2.6 Determination of Secondary Metabolites and Proximate Constituents

The aqueous extract was now subjected to standard chemical tests as described for alkaloids (Harborne, 1998); saponins (Wall *et al.*, 1954), steroids, anthraquinones, phlobatannins (Trease and Evans, 1989); cardiac glycosides, cardenolides and diololides (Sofowora, 1993); tannins and triterpenes (Odebiyi and Sofowora, 1978), phenolics and flavonoids (Awe and Sodipo, 2001). The proximate constituents of the fruit pulp and that of its formulated feed were determined using the method of Pearson (1976) for moisture and ash content. The methods described by Association of Official Analytical Chemists (A.O.A.C) (1980) was used for crude protein, fibre and lipid while carbohydrate was determined using the method described by Oyeleke (1984).

2.7 Animal Grouping and Induction of Ulcer

Albino rats (88) were acclimatized for one week and were kept in well-ventilated house conditions (temperature: $22 \pm 3^{\circ}\text{C}$; photoperiod: 12 hrs light and 12 h dark; humidity: 40–45%) with free access to rat pellets (Premier Feed Mills Company Limited, Ibadan) and tap water before the commencement of the experiment. After a week, they were weighed {initial weight (g)} and assigned into two groups: A (8 animals {Non-aspirin induced ulcerogenic rats}) and group B (80 animals {Aspirin induced ulcerogenic rats}). They were all fasted for 12 hours before ulceration was induced in the animals in group B by oral administration of 200 mg/kg body weight of aspirin for 3 consecutive days (Khalil *et al.*, 2010). Ulceration was confirmed by determining the levels of serum nitric oxide and pepsinogen according to the methods of Woo *et al* (2013) and Ilic and Spray (1966) respectively.

2.8 Composition of Diets and Animal Re-grouping

The feed ingredients were weighed out, thoroughly mixed manually until it was

homogenous. The feed were then made into pellets and packed in polythene bags, labelled and stored in the refrigerator. The composition (g/kg) of the diet is as shown in Table 1. After formulation of the diets, the proximate composition of the diets was determined as previously described for the fruit pulp. Animals in group A were designated as the control group which was the rats that were not administered aspirin and were fed with basal (non-supplemented) diet. The remaining animals in group B were randomly assigned into thirteen groups as presented in Table 2. The animals were maintained on their respective diets for a period of five weeks during which serum nitric oxide and pepsinogen concentration were re-determined again after the third and the fifth weeks of feeding.

2.9 Animal Sacrifice and Collection of Gastric Juice

The animals were fasted overnight (12 hours) to ensure complete gastric emptying and a steady state gastric acid secretion (Nair *et al.*, 2010). The procedure described by Yakubu *et al* (2008) was adopted for the animal sacrifice. Under diethyl ether anaesthesia, the veins after being slightly displaced (to prevent blood contamination by interstitial fluid) were cut with a sterile scalpel blade and 5ml of the blood collected into clean and dry centrifuge tubes. The blood was then left undisturbed to clot for 10 minutes at room temperature. The tubes were thereafter centrifuged at $1282 \times g$ for 5 minutes using Uniscope Laboratory centrifuge (Model SM800B, Surgifriend Medicals, Essex, England). The sera were later aspirated with Pasteur pipettes into dry, sample bottles and used within 12 hours of preparation for the various assay. For the collection of gastric juice, the stomach of each rat was ligated at both openings (at the lower esophageal sphincter and pyloric sphincter) and injected with 3cm^3 of distilled water to collect the gastric juice (Magaji *et al.*, 2007).

2.10 Determination of Ulcer Indices and Glycoprotein Content of the Gastric Juice

Ulcer index, volume and acidity was carried out by the procedure described by Gregory *et al.* (2009), Magaji *et al.*, 2007 and Parmar and Hennings (1964) respectively while the pH of the gastric juice was determined using digital pH meter. For the determination of the glycoproteins, the methods described by Goel *et al.* (1985), Winzler (1958), Disch and Borentreund (1950), Dische and Shettles (1948), Warren (1959) and Lowry *et al.* (1951) was used for determination of total carbohydrates, hexoses, hexosamine, fucose, Sialic acid and total protein respectively while the mucosubstances activity was expressed as ratio of total carbohydrates to total proteins.

2.11 Statistical Analysis

Data was expressed as the mean $\pm$ SEM of six determinations except for results from the proximate constituents and secondary plant metabolites of the fruit pulp which represents the mean of three replicates $\pm$ SEM. All results were statistically analyzed using one-way Analysis of Variance and Duncan's Multiple Range Test (Duncan, 1957). Differences between group means were considered significant at $p < 0.05$.

3.0 Results

Proximate composition of *Dialium guineense* fruit pulp reveals that it contains high carbohydrate content and a low content of moisture, ash, protein, lipid and fibre (Table 3). Furthermore, the result of the plant secondary metabolite constituent of the fruit pulp (Table 4) shows that alkaloids, saponins, tannins, anthraquinones, phenolic, flavonoids, triterpenes and steroids were detected while cardenolides, dienolides and cardiac glycosides were not detected. Alkaloid was the most abundant in the fruit pulp while anthraquinones were in trace amount.

Administration of aspirin significantly ($p < 0.05$) reduced the serum nitric oxide concentration (Figure 1) and significantly ($p < 0.05$) elevated the serum pepsinogen concentration (Figure 2) of rats when compared with the normal rats (not administered aspirin).

The serum nitric oxide and pepsinogen concentrations of rats maintained on *Dialium guineense* fruit pulp meal-based diet after 3 and 5 weeks of feeding following administration of aspirin are shown in Tables 5 and 6 respectively. The serum nitric oxide concentration of rats administered aspirin and maintained on the basal diet was significantly reduced ($p < 0.05$) after 3 weeks of feeding and the pattern of decrease was progressive and more pronounced after 5 weeks and this compared favourably ($p > 0.05$) with rats administered aspirin and maintained on 50% to 100% of the diet. However, the diet at 5 to 40% supplementation significantly and progressively ($p < 0.05$) increased the concentration of this metabolite in rats administered aspirin after 3 and 5 weeks of feeding and this increase was not significantly different ($p > 0.05$) from those maintained on at 30% and 40% supplementation at 3 weeks and 40% supplementation at 5 weeks of feeding as well as those administered aspirin and treated with the reference drug, Omeprazole.

Administration of aspirin significantly elevated ($p < 0.05$) the pepsinogen concentration of rats after 3 weeks of feeding as compared with control group and this increase was sustained till after 5 weeks. However, the fruit pulp meal-based diet at 5 to 40% inclusion levels significantly reversed this trend of increase. The reduction at 20% - 40% supplementation was not significantly different ($p > 0.05$) from those administered aspirin and treated with the reference drug, Omeprazole. However, the concentration of this metabolite in rats maintained on the fruit pulp meal-based diets at 50% - 100% supplementation were not significantly different ($p > 0.05$) from the animals administered aspirin and fed on basal diet.

The proximate composition of the *Dialium guineense* fruit pulp meal-based diet at 5% to 100% inclusion levels was not significantly different ($p < 0.05$) from that of the basal diet fed to the three groups of rats that were administered aspirin, not administered aspirin and those treated with the reference drug, Omeprazole (Table 7).

Table 1: Composition of the Diet

Aspirin-Induced Ulcerogenic Rats														
Ingredient	Control (+ve)	Control (-ve)	Reference drug (Omeprazole)	<i>Dialium guineense</i> fruit pulp meal-based diet										
				5%	10%	20%	30%	40%	50%	60%	70%	80%	90%	100%
Cornstarch	506	506	506	480.70	455.40	404.80	354.20	303.60	253.00	202.40	151.40	101.20	50.60	-
<i>D. guineense</i> fruit pulp	-	-	-	25.30	50.60	101.20	151.80	202.40	253.00	303.60	354.20	404.80	455.40	506.00
Cellulose	40	40	40	40	40	40	40	40	40	40	40	40	40	40
Sucrose	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Soybean	250	250	250	250	250	250	250	250	250	250	250	250	250	250
Soybean Oil	50	50	50	50	50	50	50	50	50	50	50	50	50	50
*Vitamin/ Mineral Mix	50	50	50	50	50	50	50	50	50	50	50	50	50	50
D- Methionine	4	4	4	4	4	4	4	4	4	4	4	4	4	4

* Vitamin/Mineral mix: Vitamin A 4,000,000 i.u; Vitamin D₃, 800,000 i.u.; Tocopherols, 400 i.u; Vitamin K₃ 800mg , Folacin, 200mg; Thiamine, 600mg; Riboflavin 1,800mg; Niacin, 6000mg; Calcium pathothenate, 4 mg; Biotin, 8 mg; Manganese, 30,000mg, Zinc, 20,000mg; 8,000mg; Choline chloride 80,000mg; Copper, 2,000mg; Iodine, 480mg; Cobalt, 80 mg; Selenium, 40mg; BHT, 2,500mg. Anticaking agent, 6000mg.
Unit of diet composed – g/ kg.

Table 2: Sub-grouping and the treatment of the animals

Groups	Treatments
B	Rats administered aspirin and fed normal basal diet
C	Rats administered aspirin and treated with reference drug (Omeprazole) (0.045mg/kg bodyweight) and fed normal basal diet.
D-N	Rats administered aspirin and fed 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and 100% <i>Dialium guineense</i> fruit-pulp meal-based diet respectively

Table 3: Proximate Composition of the Fruit Pulp of *Dialium guineense*

Parameters	Concentration (%)
Moisture	2.15 ± 0.01
Ash	3.38 ± 0.15
Crude Fibre	4.82 ± 0.05
Lipid	5.52 ± 0.07
Protein	5.67 ± 0.04
Carbohydrate (by difference)	78.32 ± 0.21

Values are means of three determinations ± SEM

Table 4: Secondary Metabolite Constituents of *Dialium guineense* Fruit Pulp

Secondary Metabolites	Concentration (%)
Alkaloids	0.567 ± 0.04
Tannins	0.03 ± 0.02
Anthraquinones	0.02 ± 0.01
Saponins	0.19 ± 0.04
Phenolics	0.07 ± 0.02
Flavonoids	0.06 ± 0.02
Triterpenes	0.05 ± 0.02
Steroids	0.03 ± 0.01
Cardiac glycosides	Not Detected
Cardenolides and Dienolides	Not Detected

Values are means of three determinations ± SEM

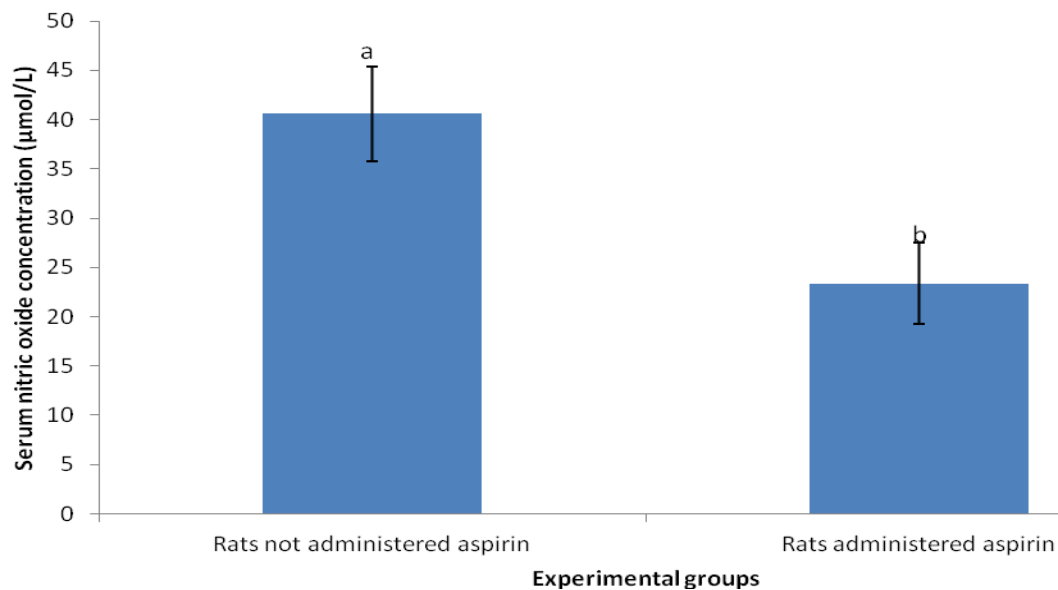


Figure 1: Serum nitric oxide concentration of rats administered aspirin
 Values are means of six determination $\pm$ SEM.
 Bars with different letters are significantly different ($p < 0.05$)

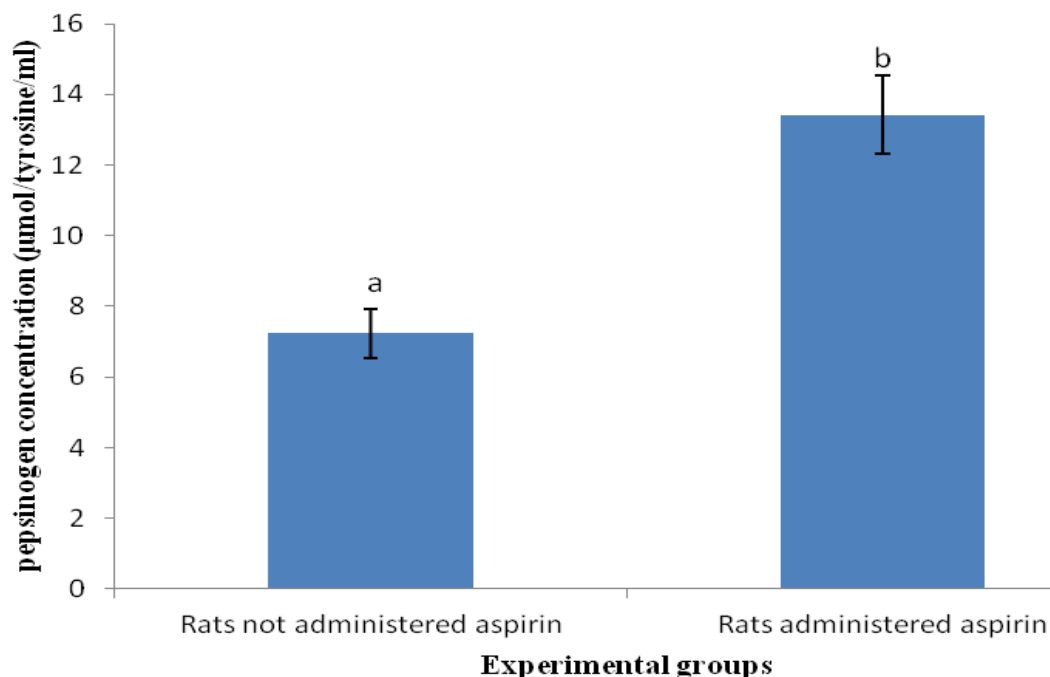


Figure 2: Serum pepsinogen concentration of rats administered aspirin
 Values are means of six determination $\pm$ SEM.
 Bars with different superscript are significantly different ($p < 0.05$)

Table 5: Effect of *Dialium guineense* fruit pulp meal-based diet on serum nitric oxide concentration ($\mu\text{mol/L}$) of rats administered aspirin after 3 and 5 weeks of feeding

Aspirin-Induced Ulcerogenic Rats														
Weeks	Rats not administered aspirin	Rats administered aspirin	Reference drug (Omeprazole)	<i>Dialium guineense</i> fruit pulp meal-based diet										
				5%	10%	20%	30%	40%	50%	60%	70%	80%	90%	100%
3	33.64 $\pm$ 1.12 ^a	23.32 $\pm$ 1.68 ^c	30.89 $\pm$ 1.01 ^a	27.81 $\pm$ 0.98 ^b	28.71 $\pm$ 2.01 ^b	26.67 $\pm$ 1.69 ^b	29.82 $\pm$ 2.77 ^a	30.48 $\pm$ 2.01 ^a	23.36 $\pm$ 1.76 ^c	23.23 $\pm$ 1.98 ^c	22.45 $\pm$ 3.08 ^c	23.43 $\pm$ 2.68	22.75 $\pm$ 2.01 ^c	21.24 $\pm$ 2.95 ^c
5	33.60 $\pm$ 2.68 ^a	23.32 $\pm$ 1.98 ^d	33.40 $\pm$ 2.91 ^a	30.49 $\pm$ 2.95 ^b	30.76 $\pm$ 0.93 ^b	32.56 $\pm$ 2.11 ^b	32.84 $\pm$ 1.95 ^a	33.31 $\pm$ 2.02 ^a	23.41 $\pm$ 2.06 ^d	23.04 $\pm$ 3.66 ^d	22.76 $\pm$ 2.04 ^d	22.85 $\pm$ 0.98 ^d	21.72 $\pm$ 1.68 ^d	21.04 $\pm$ 1.56 ^d

Values are means of 6 determination $\pm$ SEM

Values with superscript different from the control across the same row for each parameter are significant different ($p < 0.05$)

Table 6: Effect of *Dialium guineense* fruit pulp meal-based diet on serum pepsinogen concentration ($\mu\text{mol/tyrosine/ml}$) of rats administered aspirin after 3 and 5 weeks of feeding

Aspirin-Induced Ulcerogenic Rats														
Weeks	Rats not administered aspirin	Rats administered aspirin	Reference drug (Omeprazole)	<i>Dialium guineense</i> fruit pulp meal-based diet										
				5%	10%	20%	30%	40%	50%	60%	70%	80%	90%	100%
3	7.18 $\pm$ 0.98 ^a	13.38 $\pm$ 1.29 ^d	9.29 $\pm$ 1.01 ^b	11.11 $\pm$ 0.86 ^c	9.72 $\pm$ 1.01 ^b	9.27 $\pm$ 0.97 ^b	8.97 $\pm$ 1.32 ^b	8.85 $\pm$ 1.02 ^b	13.30 $\pm$ 2.03 ^d	13.35 $\pm$ 1.09 ^d	13.33 $\pm$ 0.98 ^d	13.31 $\pm$ 1.37 ^d	13.24 $\pm$ 2.01 ^d	13.56 $\pm$ 1.49 ^d
5	7.09 $\pm$ 1.68 ^a	13.44 $\pm$ 2.16 ^d	7.26 $\pm$ 1.76 ^b	10.03 $\pm$ 1.63 ^c	8.64 $\pm$ 1.03 ^b	7.83 $\pm$ 0.98 ^b	7.12 $\pm$ 1.14 ^b	7.09 $\pm$ 0.98 ^b	13.22 $\pm$ 2.01 ^d	13.55 $\pm$ 1.93 ^d	13.23 $\pm$ 1.41 ^d	13.27 $\pm$ 1.46 ^d	13.33 $\pm$ 1.62 ^d	13.49 $\pm$ 2.11 ^d

Values are means of 6 determination $\pm$ SEM

Values with superscript different from the control across the same row for each parameter are significant different ($p < 0.05$)

Table 7: Proximate Composition of the Formulated Diet

Aspirin-Induced Ulcerogenic Rats												
Parameters (%)	Rats not administered aspirin (Control), Rats administered aspirin and Omeprazole treated rats (All fed the basal diet)	<i>Dialium guineense</i> fruit pulp meal-based diet										
		5%	10%	20%	30%	40%	50%	60%	70%	80%	90%	100%
Moisture	6.35 ± 0.02 ^a	6.34 ± 0.03 ^a	6.34 ± 0.03 ^a	6.35 ± 0.02 ^a	6.34 ± 0.02 ^a	6.34 ± 0.02 ^a	6.35 ± 0.03 ^a	6.35 ± 0.01 ^a	6.35 ± 0.02 ^a	6.35 ± 0.01 ^a	6.34 ± 0.03 ^a	6.35 ± 0.03 ^a
Ash	7.85 ± 0.03 ^a	7.85 ± 0.03 ^a	7.90 ± 0.03 ^a	7.85 ± 0.04 ^a	7.95 ± 0.02 ^a	7.85 ± 0.01 ^a	7.94 ± 0.01 ^a	7.87 ± 0.04 ^a	7.86 ± 0.04 ^a	7.92 ± 0.03 ^a	7.93 ± 0.03 ^a	7.86 ± 0.02 ^a
Crude Fibre	4.15 ± 0.06 ^a	4.18 ± 0.03 ^a	4.18 ± 0.02 ^a	4.16 ± 0.03 ^a	4.15 ± 0.01 ^a	4.18 ± 0.02 ^a	4.17 ± 0.01 ^a	4.17 ± 0.04 ^a	4.16 ± 0.03 ^a	4.16 ± 0.02 ^a	4.16 ± 0.03 ^a	4.16 ± 0.02 ^a
Lipid	8.45 ± 0.03 ^a	8.42 ± 0.02 ^a	8.40 ± 0.01 ^a	8.40 ± 0.04 ^a	8.41 ± 0.03 ^a	8.44 ± 0.02 ^a	8.46 ± 0.03 ^a	8.46 ± 0.03 ^a	8.40 ± 0.02 ^a	8.40 ± 0.03 ^a	8.42 ± 0.02 ^a	8.44 ± 0.01 ^a
Protein	23.12 ± 1.02 ^a	23.10 ± 0.02 ^a	23.11 ± 0.02 ^a	23.09 ± 0.01 ^a	23.14 ± 0.01 ^a	23.11 ± 0.03 ^a	23.09 ± 0.01 ^a	23.11 ± 0.02 ^a	23.01 ± 0.01 ^a	23.10 ± 0.01 ^a	23.12 ± 0.02 ^a	23.12 ± 0.01 ^a
Carbohydrate	50.09 ± 0.06 ^a	50.10 ± 0.06 ^a	50.08 ± 0.05 ^a	50.06 ± 0.06 ^a	50.07 ± 0.06 ^a	50.08 ± 0.05 ^a	50.09 ± 0.05 ^a	50.08 ± 0.05 ^a	50.07 ± 0.02 ^a	50.08 ± 0.05 ^a	50.09 ± 0.04 ^a	50.09 ± 0.03 ^a

Values are means of 3 determination ± SEM

Values with the same superscript a, across the same row for each parameter are not significant different (p>0.05)

Administration of aspirin to rats fed on basal diet resulted in significant increase ($p < 0.05$) in the ulcer index, volume and acidity of the gastric juice (Table 8). However, the fruit pulp meal-based diet was able to reduce significantly ($p < 0.05$) the ulcer index at inclusions of 5% to 40% although this amelioration was significantly different ($p < 0.05$) from rats treated with the reference drug, Omeprazole. In addition, there was a significant reduction ($p < 0.05$) in the ulcer index of rats fed on 5% and 10% inclusions of the fruit pulp when compared with those fed on 20%, 30% and 40% inclusions. The diet significantly reduced ($p < 0.05$) the volume and quantity of the gastric juice at 5% to 40% inclusion with rats fed on 20% - 40% fruit pulp supplementation not significantly different ($p > 0.05$) from those administered aspirin and treated with the reference drug, Omeprazole. In contrast, there was no significant difference ($p > 0.05$) in the values of these parameters in rats administered aspirin and fed on basal diet and those fed on 50% to 100% inclusions of fruit pulp meal-based diet.

A significant reduction ($p < 0.05$) was noted in the pH of the gastric juice after administration of aspirin but the fruit pulp meal-based diets at 5% - 40% inclusions was able to increase ($p < 0.05$) significantly the pH and interestingly, those fed on 30% and 40% supplementation of the diet not significantly different ($p > 0.05$) from those treated with the reference drug, Omeprazole. Like the other parameters of acid secretion, the pH of the gastric juice of rats fed on 50% - 100% inclusions of the fruit pulp were not significant difference ($p > 0.05$) from those administered aspirin and were not treated.

Administration of aspirin to rats significantly reduced ($p < 0.05$) the hexose, hexosamine, fucose, sialic acid, total carbohydrate concentrations and total carbohydrate: protein ratio whereas it significantly increased ($p < 0.05$) the total protein concentration. Supplementations of *Dialium guineense* fruit pulp at 5% - 40% significantly elevated the concentrations of hexose, hexosamine, fucose, sialic acid, total carbohydrate concentrations and total carbohydrate: protein ratio and significantly reduced ($p < 0.05$) the total protein concentration (Table 9). For the hexose, hexosamine concentrations and total carbohydrate/protein

ratio, the most profound increase was exhibited in rats fed on 30% and 40% *Dialium guineense* fruit pulp meal-based diet while for the fucose, sialic acid and total carbohydrate concentrations, this increase was found in rats fed on 40% of the diet and this were not significantly different from those of the rats not administered aspirin and those treated with the reference drug, Omeprazole. Furthermore, the total p rotein content of rats maintained on 40% inclusion of *Dialium guineense* fruit pulp was decreased to values that compared favourably with rats not administered aspirin and those treated with Omeprazole (Table 9).

4.0 Discussion

Proximate analysis of a food gives an estimation of the amount of carbohydrate, lipid, crude protein and in fat food. The high carbohydrate content of the fruit pulp of *Dialium guineense* in comparison with other constituents may explain its sweet taste and this high content is within the range stated in the literature (Odukoya *et al.*, 1996).

Carbohydrate, especially glucose is an energy source important for the maintenance of cellular function and biochemical reactions and this has been reported to diminish acid-back diffusion and lipid peroxidation in the gastric mucosal and elevate the mucus level by regeneration (Hung and Neu, 1997). Most enzymes are proteinous in nature (Nelson and Cox, 2005).

Pepsin, a protein, which is a principal enzyme of the gastric juice is derived from chief cells (Singh and Guha, 2012) and is responsible for the proteolytic activity of gastric juice and the higher the stimulation of its production by the consumption of very proteinous food, the higher the induction of ulcer in the gastrointestinal tract. Therefore, low proteinous foods are recommended to reduce its stimulation. The low protein content of *Dialium guineense* fruit pulp is in line with the proposition of Dekkers (1956) in which he hypothesized a low-protein and high carbohydrate diet in patient with gastric ulcer. Fibre is a constituent of food which is reported to accelerate ulcer healing by increasing the secretion of gastric mucus which protects the gastric mucosal against the damaging effect of

Table 8: Effect of *Dialium guineense* fruit pulp meal-based diet on acid secretory parameters of rats administered aspirin

Aspirin-Induced Ulcerogenic Rats														
Parameters	Rats not administered aspirin	Aspirin-treated rats	Omeprazole-treated rats	<i>Dialium guineense</i> fruit pulp meal-based diet										
				5%	10%	20%	30%	40%	50%	60%	70%	80%	90%	100%
Ulcer Index (mm ²)	-	5.62 ± 0.23 ^d	1.62 ± 0.17 ^a	3.62 ± 0.16 ^c	3.46 ± 0.21 ^c	2.25 ± 0.16 ^b	2.16 ± 0.52 ^b	2.18 ± 0.31 ^b	5.72 ± 0.23 ^d	5.85 ± 0.13 ^d	5.64 ± 0.17 ^d	5.72 ± 0.11 ^d	5.58 ± 0.08 ^d	5.82 ± 0.12 ^d
Volume of gastric juice (ml)	4.27 ± 0.09 ^a	6.07 ± 0.12 ^d	4.60 ± 0.04 ^b	5.17 ± 0.15 ^c	5.02 ± 0.02 ^c	4.87 ± 0.10 ^b	4.66 ± 0.37 ^b	4.67 ± 0.37 ^b	6.02 ± 0.07 ^d	5.94 ± 0.12 ^d	6.07 ± 0.20 ^d	6.19 ± 0.24 ^d	6.14 ± 0.10 ^d	6.12 ± 0.14 ^d
Gastric juice acidity (ml)	4.57 ± 0.22 ^a	14.73 ± 0.84 ^d	7.20 ± 0.19 ^b	10.34 ± 0.57 ^c	8.05 ± 0.91 ^b	7.40 ± 0.49 ^b	7.36 ± 0.58 ^b	7.36 ± 0.48 ^b	14.71 ± 1.19 ^d	14.46 ± 0.50 ^d	14.62 ± 1.09 ^d	15.34 ± 0.38 ^d	14.07 ± 0.73 ^d	14.96 ± 1.09 ^d
pH of gastric juice	4.69 ± 0.02 ^a	2.32 ± 0.02 ^d	4.20 ± 0.22 ^b	3.39 ± 0.04 ^c	3.52 ± 0.16 ^c	3.69 ± 0.14 ^c	4.16 ± 0.08 ^b	4.15 ± 0.20 ^b	2.53 ± 0.11 ^d	2.48 ± 0.10 ^d	2.47 ± 0.23 ^d	2.46 ± 0.11 ^d	2.51 ± 0.07 ^d	2.47 ± 0.09 ^d

Values are means of 6 determination ± SEM

Values with superscript different from the control across the same row for each parameter are significant different (p < 0.05)

Table 9: Effect of *Dialium guineense* fruit pulp meal-based diet on the glycoprotein concentration in the gastric juice of rats administered aspirin

Aspirin-Induced Ulcerogenic Rats								
<i>Dialium guineense</i> fruit pulp meal-based diet								
Parameters	Rats not administered aspirin	Aspirin-treated rats	Omeprazole-treated rats	5%	10%	20%	30%	40%
Hexose (µg/ml)	311.53 ± 5.06 ^a	187.03 ± 6.69 ^c	308.80 ± 5.93 ^a	289.87 ± 1.80 ^b	297.26 ± 6.63 ^b	295.25 ± 5.55 ^b	303.08 ± 5.51 ^a	302.17 ± 8.13 ^a
Hexosamine (µg/ml)	175.25 ± 4.07 ^a	127.25 ± 3.11 ^c	172.51 ± 3.45 ^a	160.42 ± 5.31 ^b	157.77 ± 2.99 ^b	160.78 ± 3.84 ^b	162.84 ± 2.69 ^a	166.58 ± 2.82 ^a
Fucose (µg/ml)	115.07 ± 3.35 ^a	49.36 ± 3.81 ^c	107.65 ± 3.55 ^{ab}	89.82 ± 0.42 ^b	93.38 ± 4.06 ^b	96.70 ± 2.86 ^b	102.94 ± 2.90 ^b	109.48 ± 2.34 ^a
Sialic acid (µg/ml)	70.40 ± 1.55 ^a	29.77 ± 1.11 ^c	62.15 ± 2.51 ^a	50.05 ± 2.76 ^b	54.26 ± 2.60 ^b	353.16 ± 3.38 ^b	56.95 ± 2.32 ^b	58.72 ± 2.35 ^a
Total Carbohydrate (TC) (µg/ml)	677.24 ± 6.40 ^a	359.93 ± 28.24 ^d	653.70 ± 7.78 ^a	584.15 ± 4.85 ^c	610.48 ± 9.76 ^b	613.88 ± 1.77 ^b	625.81 ± 11.92 ^b	649.93 ± 9.77 ^a
Total Protein (TP) (µg/ml)	310.73 ± 6.70 ^a	415.29 ± 7.73 ^d	321.47 ± 4.69 ^a	347.53 ± 4.92 ^c	333.09 ± 5.47 ^b	328.23 ± 6.92 ^b	330.84 ± 6.55 ^b	322.70 ± 4.01 ^a
TC:TP	2.18 ± 0.07 ^a	0.87 ± 0.59 ^d	1.97 ± 0.04 ^a	1.68 ± 0.04 ^c	1.83 ± 0.06 ^b	1.87 ± 0.04 ^b	1.89 ± 0.03 ^a	1.95 ± 0.04 ^a

Data are means of six determinations ± SEM. Values with superscripts different from the control across each column for each parameter are significantly different (p < 0.05).

acid and pepsin (Achoba *et al.*, 1992). The ash content of a food sample gives an insight into the mineral composition of that food substance (Achoba *et al.*, 1992) and some of these minerals might serve as antioxidants. Extensive researches have proven that antioxidants might be effective not only in protecting against gastric mucosal, but also inhibiting progression of gastric ulcer (Dekkers *et al.*, 1996). A high fat intake have been implicated in the pathogenesis of gastric ulcer due to the fact that it increased consumption results in delayed gastric emptying leading to the production of gastric acid secretion. The values obtained for fibre, ash and lipid of *Dialium guineense* fruit pulp were higher than those reported by Adepoju (2009) and this might have resulted from climatic, geographical and seasonal variations. The moisture content of any food is an index of its water activity (Frazier and Westeff, 1978) and is used as a measure of stability and susceptibility to microbial contamination (Scott, 1980). The low moisture content of the fruit pulp is in close agreement with the value reported in literature (Dike, 2010) and this implies that the sample of the fruit pulp of *Dialium guineense* may have a long shelf-life due to its low moisture content.

The medicinal value of plants lies in its bioactive secondary metabolite constituents that produce definite physiological action (Akinmoladun *et al.*, 2007). The secondary metabolites found in the fruit pulp of *Dialium guineense* are known to affect the integrity of mucus membranes. They have also been reported to have antioxidant properties (Annan and Houghton, 2008) and promote prostaglandin secretion (Watcho *et al.*, 2010). Alkaloids, the constituent with the highest percentage found in the fruit pulp are known to inhibit lesions produced by aggressive agents and alter gastric acid secretion through increase of luminal gastric output of basal bicarbonate and pH (Wan, 1993). Tannins have astringent properties which have been reported to hasten the healing of wounds and inflamed mucous membrane (Asuzu and Onu, 1990). They are also known to protect the outermost layer of mucosa and to render it less permeable and more resistant to chemicals and mechanical injury or irritation and thus prevent ulcer development (Asuzu and Onu, 1990). The biological functions of

flavonoids include protection against allergies, platelet aggregation and ulcer (Okwu and Okwu, 2004). Flavonoids have also been reported to play a role in stabilizing the antioxidant status of the gastric mucosa and are also known to have anti-inflammatory activity and protect the gastric mucosa against a variety of ulcerogenic agents in different mammalian species (Harbone and Williams, 2000). Plants containing flavonoids have been found to be effective in preventing this kind of lesion, mainly because of their antioxidant properties (Cody *et al.*, 1986; Harbone and Williams, 2000). Flavonoids and tannins are phenolic compounds which are a major group of compounds that act as primary antioxidants (Polterait, 1997); capable of scavenging peroxy radicals because these compounds contain an OH group linked with the aromatic ring and thus may possess potential antioxidant (Karakaya, 2004) and antiulcer (Bandyopadhyay *et al.*, 2002) activities. The presence of these metabolites in the fruit pulp may therefore among others be responsible for the acclaimed antiulcer activities, thus supporting its use for the healing of ulcer.

The experimental model (aspirin-induced ulceration) employed in this study is one of the most commonly used to study ulceration in the gastrointestinal tract in animals (Nafeeza *et al.*, 2002; Goel *et al.*, 1986) and has gained wide acceptance. Non-Steroidal anti-inflammatory drugs (NSAIDs) especially aspirin represents one of the most widely used classes of therapeutic agents, but their tendency to cause ulceration and bleeding in the gastrointestinal tract is a major problem (Wallace, 1997).

Aspirin causes ulceration mainly by the suppression of cyclooxygenase which results in inhibition of prostaglandin synthesis (Wallace and Tigley, 1995). Prostaglandins are known to play crucial roles in mechanisms required for inhibiting acid secretion by promoting the secretion of mucus and strengthening resistance of the mucosal barrier to back diffusion of acid from the gastric lumen into the sub-mucosal tissues, increasing the mucosal blood flow, maintaining the cellular integrity of the mucosa (Hanan *et al.*, 2010) amongst many others. Therefore, inhibition of prostaglandins by aspirin consequently leads to inhibition of several compounds of gastric mucosal defense

mediated by this substance which include mucus and bicarbonate secretion, nitric oxide stimulation, blood flow, epithelial cell turnover etc. and activation/ stimulation of aggressive factors such as pepsinogen, acid, pepsin, gastrin and histamine (Alarcon-de *et al.*, 1994).

Inhibition of nitric oxide stimulation by depletion of prostaglandin synthesis has been implicated in ulceration (Brown *et al.*, 1992). Nitric oxide is a crucial mediator of gastrointestinal mucosal defense and repair (Muscara and Wallace, 1999) and its inactivation has been implicated in ulceration (Brown *et al.*, 1992). Ulceration in experimental models has been shown to be healed at a greatly accelerated rate when they were treated daily with nitric oxide donors (Elliott *et al.*, 1995). Its secretion has correlation with that of prostaglandin and evidence exists that nitric oxide is one of the factors responsible for the secretion of gastric mucus (Brown *et al.*, 1992). Nitric oxide may also modulate mucosal repair by its ability to enhance collagen deposit by fibroblast to stimulate angiogenesis (Schaffer *et al.* 1996; Jadeski and Lala, 1999). Another mechanism through which nitric oxide may accelerate healing of the gastrointestinal ulcer is by contributing to the maintenance of blood flow at the margin of the lesion. In the stomach, the healing of ulcer depends on proliferation and differentiation of epithelial cells at the ulcer margin. This region receives a high rate of blood flow, and it has been clearly shown that a reduction of blood flow to the ulcer margin results in a retardation of ulcer healing (Hirose *et al.*, 1991; Iwata and Leung, 1995). Therefore, nitric oxide is a crucial defensive factor in ulceration and its suppression by aspirin in this study confirmed that ulceration has been established. This is in accordance with the findings of Ashraf and Adel (2009) who reported a similar inhibitory effect on nitric oxide level in aspirin-induced ulceration in rats.

Mucus, one of the defensive factors in the gastrointestinal mucosa acts as a lubricant to reduce physical abrasion of the mucosa and participates in the protection of the mucosa from the aggressive factors induced by aspirin intake. An increase in mucus thickness is a normal defensive response and Brown *et al.* (1992) has reported that nitric oxide is an important

regulator of mucus secretion and its effects are produced *via* stimulation of guanylyl cyclase in the epithelial cell.

Pepsinogen is an inactive precursor of pepsin and at pH 6.0 or below, it is auto catalytically converted to pepsin (Selvamathy *et al.*, 2010). The activities and concentrations of these enzymes are largely dependent on gastric acidity and since ulcer induction by aspirin has been linked to increase in acid secretion, determination of pepsinogen concentration is widely used as one of the indirect tests for the determination of gastric function in aspirin-induced ulceration in the blood (Singh and Guha, 2012). Therefore, the increase in pepsinogen concentration after the administration of aspirin when compared to the rats that were not administered aspirin correlates with the report of Singh and Guha (2012) who reported that increased pepsinogen secretion is linked with increased acid secretion since acidification is required to activate it to pepsin.

Proximate analysis refers to the determination of the major constituents of a feed (quantitatively) and it is used to assess if a feed is within its normal compositional parameters. It partitions nutrients in feed into six components which include water, ash, crude protein, crude lipid, crude fibre and Nitrogen free extract (majorly soluble carbohydrate) (Atteh, 2002). The percentage composition of the formulated diet as depicted in Table 5 indicates that the constituents of both the control and experimental diets were not quantitatively different and this implies that any differences which may eventually emanate from the observation of rats fed on the feed samples will be attributed to the quality of the feeds. This finding is similar to that reported by Muhammad *et al* (2006).

Acid secretory parameters and its related indices give a reflection of the degree of ulceration in the gastrointestinal mucosal. Acid is considered an important factor in the development of acute and chronic gastric mucosal lesions (Shakeerabanu, 2011). It is evident from this study that aspirin was able to elevate the level of gastric acid in the juice of rats. This may be due to the suppression of defensive factors of the gastric mucosal especially prostaglandins thereby boosting the aggressive factors. These findings agree with

those of Scheiman (1992) whose work revealed that gastric acid plays an important permissible role in NSAID associated mucosal injury. Therefore, suppression of gastric acid by a variety of pharmacological means provides effective and rapid healing of ulcer (Walsh and Peterson, 1995).

The fruit pulp of *Dialium guineense* might have been able to perform this function probably by boosting some defensive factors especially prostaglandins, a key molecule that stimulates the secretion of mucin, bicarbonates, glycoproteins and inhibit the activity of $H^+ / K^+ - ATPase$ that is responsible for the pumping of the gastric acid (Di Carlo *et al.*, 1999). Flavonoids speculated by Borelli and Izzo (2000) that protects the gastric mucosa from damage by increasing the mucosal prostaglandin content thereby reducing the gastric acidity may as well be responsible for the antiulcer activity of the plant in the present study. Acid reducing property has been related to several anti-ulcer drugs (Borelli and Izzo, 2000).

The ulcer index is a reflection of the level of gastric acidity in the mucosal (Magaji *et al.*, 2007). It is evident from this study that aspirin created an ulcer lesion in rats administered aspirin which were absent in the rats that were not treated with aspirin. This index of lesion was reduced with treatment with the fruit pulp. The finding with respect to ulcer lesion in the present study correlates well with reduction in gastric acidity. This finding is similar to that reported by Selvamathy *et al* (2010) on the correlation between the ulcer index and gastric acid secretion.

The volume of acid present in gastric secretion which encompasses HCl, pepsinogen, protein and other aggressive factors reflects the concentration of the acid (Inas *et al.*, 2011) and exposure of unprotected lumen of the mucosa to accumulating acid (Olsen, 1988). In the present study, the volume of gastric juice correlated well with significant rise in acid secretion following the administration of aspirin. The reduction in the volume of the juice in the animals maintained on the *Dialium guineense* fruit pulp meal-based diet is a reflection of the decrease in gastric acid secretion. This result is similar to the findings of Olsen (1988). Furthermore, this finding on volume of juice in relation to acid

secretion also correlates with the pH values which decreased as the concentration of hydrogen ions in the juice increased. The low pH in rats administered aspirin is also a reflection of an increase in gastric acid secretion and the ability of the fruit pulp to increase the pH significantly is also similar with that previously reported by Olsen (1988).

Glycoproteins are proteins that contain oligosaccharide chains (glycans) covalently attached to their polypeptide side-chains. The carbohydrate is attached to the protein in a co-translational or posttranslational modification. This process is known as glycosylation (Selvamathy *et al.*, 2010). These glycoproteins are constituents of the gastric mucus and are important for their specific properties such as gel formation and viscosity. The increase in these mucopolysaccharides (fucose, hexose, hexosamine and sialic acid) strengthens the mucosal thereby preventing it from the effect of acid and pepsin. This can therefore prevent ulceration or reduce ulceration (Kumar *et al.*, 2010). The decrease in the levels of these mucopolysaccharides in rats administered aspirin could be a consequence of an imbalance in the defensive factors leading to elevation in acid secretion thereby causing ulceration. However, the fruit pulp of *Dialium guineense* boost the levels of these glycoproteins as evident from the increase in mucus secretion which prevented mucosal injury (Goel *et al.*, 1994).

The decrease in ratio of total carbohydrate to total protein (TC: TP) of rats administered aspirin is a reflection of mucin activity which was evident from the reduced level of individual mucopolysaccharides (Goel *et al.*, 1994). The fruit pulp of the plant exhibited ulcer healing at 5% - 40% inclusions by boosting this ratio (TC:TP). The increase in protein content of the juice following the administration of aspirin resulted in a decrease in TC:TP ratio which indicates damage to the gastric mucosa. Consequently, the plasma protein may leak into the gastric juice. However, the decrease in protein content of the gastric juice of the ulcerogenic animals treated with *Dialium guineense* fruit pulp is an indication of decreased leakage from the mucosal cells suggesting mucosal resistance (Jainu *et al.*, 2002).

The present study concludes that the fruit pulp of *Dialium guineense* exhibits anti-ulcerogenic activity as evident in the reduction in gastric acidity, gastric volume, ulcer index, pepsinogen, and total protein concentration. This is further supported by increase in serum nitric oxide, total carbohydrate concentration of the glycoproteins in the gastric juice and pH. These findings further provides scientific evidence to the acclaimed folkloric use of *Dialium guineense* fruit pulp in the management of ulcer.

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