



<https://doi.org/10.6084/m9.figshare.12295853>

<https://doi.org/10.7910/DVN/KM10A2>

Effect of *Dialium guineense* Fruit Pulp Meal-based Diet on Haematological parameters, Lipid profile and Organ/body weight ratio of Rats

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Abstract

Haematological, lipid profile and organ/body weight ratio are some of the biochemical parameters used in assessing the toxicity of a food/drug. The study aimed at determining these parameters in albino rats fed the fruit pulp of *Dialium guineense* at 5% - 40% inclusion levels. 36 rats of both sexes were grouped into 6 (A – F). A served as the control while B – F were maintained on *Dialium guineense* fruit pulp meal-based diet at 5% - 40% supplementation respectively for five weeks. Haematological and lipid Profiles were determined using standard methods while Organ/body weight ratio was expressed in percentage. The study revealed no alteration in almost all the haematological parameters of the test groups when compared with the control except for lymphocytes in which a significant increase ($p < 0.05$) ($50.72 \pm 1.73\%$) was observed at 40% supplementation while for neutrophils, significant increase and decrease ($p < 0.05$) was observed in the group fed on 30%; 40% diet (increase) ($47.68 \pm 2.13\%$, $48.11 \pm 2.20\%$) and 5% diet (decrease) ($41.68 \pm 0.99\%$) respectively. For the lipid profile, a significant increase was observed at all inclusion levels in the total cholesterol and high density lipoprotein concentration while there was a significant decrease ($p < 0.05$) in the low density lipoprotein concentration, triglyceride and atherogenic index at all inclusion levels of the fruit pulp. For the Organ body weight ratio, no significant difference ($p > 0.05$) was observed in all the groups. The results suggest that the diet might not exhibit structural toxicity on selected organs but portray indications of minute functional toxicity which are still within the tolerable range.

Keywords: Haematological parameters, *Dialium guineense*, Lipid profile, Atherogenic index.

Introduction

Reference ranges of physiological parameters can be useful for the evaluation of the state of health in specimens of the species as well as diagnostics and prevention of diseases (Tomenendalova, 2014). Haematology refers to the study of the numbers and morphology of the cellular elements of the blood and the use of these results in the diagnosis and monitoring of disease with the respective damage to blood (Merck, 2012, Onyeyili *et al.*, 1992, Togun *et al.*, 2007). Haematological parameters are used to assess the status of the blood and are related to the blood and blood-forming organs (Stenesh, 1975). Their elevated or decreased level has various clinical implications. They include Red blood Cell Counts, Haemoglobin, Haematocrit, Mean Corpuscular haemoglobin, Mean

Corpuscular Haemoglobin Concentration, White blood Cell Count, Platelet Counts *e.t.c.* (NseAbasi, 2014).

Blood is a vital substance made up of red (erythrocytes) and white blood cells (leukocytes) suspended in a liquid called blood plasma. The blood of mammals contains many proteins which are involved in blood clotting. Blood circulates around the body in arteries and veins; and acts as a transport system for many substances including oxygen, amino acids (proteins), lipids (fats), sugar, glucose (carbohydrate), hormones and waste products (ammonia and carbon dioxide). Blood act as a pathological reflector of the status of the exposed animals to toxicants and other conditions (NseAbasi, 2014).

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Blood is a vital substance made up of red (erythrocytes) and white blood cells (leukocytes) suspended in a liquid called blood plasma. The blood of mammals contains many proteins which are involved in blood clotting. Blood circulates around the body in arteries and veins; and acts as a transport system for many substances including oxygen, amino acids (proteins), lipids (fats), sugar, glucose (carbohydrate), hormones and waste products (ammonia and carbon dioxide). Blood act as a pathological reflector of the status of the exposed animals to toxicants and other conditions (NseAbasi, 2014).

Serum is the fluid portion of the blood obtained when the blood is allowed to clot in the absence of an anticoagulant (Yakubu, 2006). It is free of clotting proteins but contains the clotting metabolites that result from the clotting process. It is a cleaner sample typically free of cells and platelets because they are trapped in the fibrin meshwork of the clot. The serum is responsible for the homeostasis process within the cell (Ganong, 2001). It contains proteins like albumin and globulin (Murray *et al.*, 2000), and immense number of ions, inorganic and organic molecules that are in transit to other parts of the body and thus aid transport of other substances (Ganong, 2001). It also contains water, proteins, glucose, lipids, amino acids, enzymes, hormones, antigens and urea (Ganong, 2001). Lipids are fatty substances that are normally insoluble in an aqueous environment but are solubilised and transported in the plasma as water-soluble macromolecular complexes known as lipoproteins (Styrer, 1995). Lipids found in the serum that are of diagnostic importance include cholesterol, triglycerides, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol and phospholipids (Nelson and Cox, 2000).

An important requirement in toxicological experiments is the ability to assess the effects of xenobiotics on specific organs. For many organs, this is done through macroscopic examination of the organs, measuring organ weight, and histopathological examination of the tissue (Bailey *et al.*, 2004). Organ weight can be the most sensitive indicator of an effect of an experimental compound, as significant differences in organ weight between treated and untreated (control) animals may occur in the absence of any morphological changes (Bailay *et al.*, 2004). The organ-body weight ratio is the ratio of the organ to the body weight of an animal (Green, 1980) and it is used in predicting clinical conditions. It is a 'marker' of cell constriction and inflammation (Moore and Dalley, 1999) and is normally investigated to determine whether the size of the organ has changed in relation to the weight of the whole animal (Adebayo *et al.*, 2003) and this could reflect the state of the organ and consequently its function.

Medicinal plants were considered by several researchers to form an important component of the natural wealth of the country (Egharevba and Ikhatua, 2008). In fact, most

plants produce a diverse range of bioactive molecules making them rich sources of medicinal compounds which have continued to play a dominant role in the maintenance of human health since ancient times. These bioactive molecules termed phytochemicals are non-nutritive plant chemicals that possess the curative properties ((Egharevba and Ikhatua, 2008).

Dialium guineense (Wild) belongs to the family of *Fabaceae*. It is known as Velvet or Black tamarind (English), Awin (Yoruba), Icheku (Igbo), Tsamiyar kurmi (Hausa) (Aiyeloja and Bello, 2006). It is a woody plant that grows up to 15 m high in the rain forest region of West Africa (Okegbile and Taiwo, 1990). It has densely leafy crown, smooth greyish bark. Leaves are hairy and the flowers are usually whitish while the fruits are less circular and flattened. The small black velvet fruits are very conspicuous and distinctive. Fruits usually abundant, more or less circular and flattened, but sometimes almost globose, up to 2.5 cm in diameter, densely velvety, black; each fruit with a stalk about 6 mm long with a little collar near the apex, with a brittle shell enclosing 1 seed (or exceptionally 2), embedded in a dry, brownish, sweetly acidic, edible pulp (Aiyeloja and Bello, 2006). The fruit pulp which is yellowish-red in colour has a sweet-sour, astringent flavour similar to baobab but sweeter than it and it is eaten raw by man and animal.

The decoction of the bark of *Dialium guineense* is prescribed for stomach-ache and mouth wash (Gills, 1992). The leaves of *Dialium guineense* has also been reported for the treatment of cough and fever (Gills, 1992).. Adjanohoun *et al* (1989) reported the anti-diarrhoeal activity of the aqueous root extract of *Dialium guineense*. Odukoya *et al* (1996) also reported the anti-microbial activity of the ethanolic extract of the leaves of *Dialium guineense* plant. The fruit pulp of the plant is claimed locally to have anti-ulcerogenic potential and this has been scientifically authenticated by Oyegoke and Oladiji (2014) with the determination of the secondary metabolite constituents of the aqueous extract, proximate constituent of the fruit pulp and the formulated feed.

However, after authenticating the efficacious use of the fruit pulp of this plant as an anti-ulcer agent, we realize that there is dearth of information on its toxicological implications in open scientific literature; therefore, there is need to provide information to fill this lacuna. Therefore, the overall objective is to evaluate the safety of *Dialium guineense* fruit pulp in rats as it relates to haematological parameters, lipid profile and Organ body weight.

Materials and Methods

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.

Laboratory Animals

Thirty six albino rats (*Rattus norvegicus*) of both sexes ($167.09 \pm 6.78\text{g}$, 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.

Feed Ingredient

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.

Assay kits, Chemicals and Reagents

Assay kits for Cholesterol, Triglyceride, High and Low Density lipoprotein, chemicals and reagents used for this research were of analytical grade and were obtained from Randox laboratories Co-Atrim, United Kingdom.

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.

Composition of Diets

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 of the diet is shown in Table 1.

Table 1: Composition of the Diet

Ingredient	<i>Dialium guineense</i> fruit pulp meal-based diet (%)					
	Control (Basal Diet)	5	10	20	30	40
Corn starch	506	480.70	455.40	404.80	354.20	303.60
<i>D. guineense</i> fruit pulp	-	25.30	50.60	101.20	151.80	202.40
Cellulose	40	40	40	40	40	40
Sucrose	100	100	100	100	100	100
Soybean	250	250	250	250	250	250
Soybean Oil	50	50	50	50	50	50
Vitamin/Mineral	50	50	50	50	50	50
D-Methionine	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, Folicin, 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.

Animal Grouping

Albino rats (36) were acclimatized for one week and were kept in well-ventilated animal house conditions (temperature: $22 \pm 3^\circ\text{C}$; photoperiod: 12hrs light and 12hrs dark; humidity: 40 – 45%) with free access to rat pellets (Premier Feed Mills Company Limited, Ibadan,

Nigeria) and tap water before the commencement of the experiment. After a week of acclimatization, they were weighed {initial weight (g)} and assigned into six groups of six rats each (Table 2):

Table 2: Animal grouping based on Feed Composition

Group	Feed Composition
A	Basal (Non-Supplemented) diet
	<u>Percentage Inclusion of <i>Dialium guineense</i> Fruit Pulp (%)</u>
B	5
C	10
D	20
E	30
F	40

The animals were maintained on their respective diets for a period of five weeks before sacrifice.

Animal Sacrifice, Preparation of Serum and Excision of the Organs of Study

Animals were weighed before sacrifice (Final weight). 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 scapel blade and 5ml each of the blood was collected into clean and dry centrifuge tubes and heparinized sample bottles respectively. The blood collected into the centrifuge tube was left undisturbed to clot for 10 minutes at room temperature after which they 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 (non-heparinized) bottles and used within 12 hours of preparation for the lipid profile assay. The animals were quickly dissected and the liver and kidney excised, cleaned and weighed.

Determination of haematological parameters

The blood collected into the heparinised sample bottles was used for the determination of haematological parameters. The Automated Haematological Analyser, Sysmex, KX- 21 (Japan) was used to determine the levels of haemoglobin (Hb), red blood cells (RBC), Packed cell volume (PCV), Mean cell haemoglobin (MCH), Mean cell haemoglobin concentration (MCHC), Mean cell volume (MCV) and White blood cell (WBC). The Stromatolyser counts the red cells, lyses them to release the haemoglobin and estimates this concentration. The machine assumes that all nucleated cells are white cells and therefore counts all as white cells into their different forms i.e. lymphocytes and neutrophils, but will not differentiate between eosinophils, monocytes and basophils and as such, they are recorded as mixed. It lyses the white blood cells

based on the size of the nucleus and counts the number of the white cells.

Determination of serum lipid profile

The total cholesterol concentration was determined by colorimetric technique as described by Allain *et al.* (1974). Triglycerol concentrations in the serum were determined as described by Searcy (1969). HDL-Cholesterol was determined by a colorimetric technique described by Burstein and Mortin (1969). The serum low-density lipoprotein cholesterol was assayed using the polyvinyl sulphate (PVS) reaction as described by Demacker *et al* (1984). The atherogenic index was computed using the method described by Ng *et al* (1997). The computation was done adopting the expression LDL -Cholesterol/ HDL -Cholesterol.

Organ-body weight ratio (Expressed in percentage)

The animals were weighed before sacrifice. The organs of interest were excised and weighed after blotting each in blotting paper to remove blood and water. The weights were noted and the organ-body weight ratio was composed from the expression:

$$\text{Organ-Body Weight Ratio} = \frac{\text{Weight of the organ} \times 100}{\text{Weight of the animal}}$$

2.12 Statistical analysis

Data was expressed as the mean ± SEM of six determinations. All results were statistically analyzed using one-way ANOVA and Duncan’s Multiple Range Test (DMRT) (Duncan, 1957). Differences between group means were considered significant at p < 0.05.

Results

The effect of *Dialium guineense* fruit pulp meal-based diet on the haematological profile of rats is presented in Table 3. There was no significant difference (p > 0.05) in the red blood cells (RBC), haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) of rats fed on *Dialium guineense* fruit pulp meal-based diet at all levels of inclusions (5% -40%) when compared with the rats maintained on basal diet (control) group. Although, there were no significant difference (p > 0.05) in the white blood cell (WBC) at all inclusion levels of the fruit pulp meal-based diet, a significant decrease (p <0.05) was observed in the neutrophil count at 5% inclusion level while a significant increase (p < 0.05) was observed at 30% and 40% inclusion levels. Likewise, a significant increase (p < 0.05) in the lymphocyte count was also noted at 40% inclusion level of the fruit pulp.

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Table 3: Effect of *Dialium guineense* fruit pulp meal-based diet on the haematological profile of rats

Parameters	Treatment groups					
	Control	5% D.g	10% D.g	20% D.g	30% D.g	40% D.g
RBC (10 ⁶ /uL)	7.07 ± 0.13 ^a	6.85 ± 0.34 ^a	6.97 ± 0.24 ^a	6.86 ± 0.24 ^a	6.91 ± 0.65 ^a	6.86 ± 0.35 ^a
Hb (g/dl)	14.79 ± 0.04 ^a	14.55 ± 0.05 ^a	14.57 ± 0.04 ^a	14.66 ± 0.08 ^a	14.77 ± 0.08 ^a	14.83 ± 0.13 ^a
PCV (%)	39.15 ± 0.26 ^a	38.81 ± 0.79 ^a	39.16 ± 0.77 ^a	39.28 ± 0.52 ^a	38.93 ± 1.44 ^a	38.75 ± 1.11 ^a
MCV (fl)	62.49 ± 0.28 ^a	61.47 ± 1.44 ^a	62.18 ± 1.60 ^a	61.82 ± 1.85 ^a	61.88 ± 0.26 ^a	61.72 ± 0.21 ^a
MCH (pg)	17.10 ± 0.11 ^a	17.04 ± 0.27 ^a	16.94 ± 0.53 ^a	16.98 ± 0.84 ^a	16.99 ± 0.59 ^a	17.14 ± 0.34 ^a
MCHC (g/dl)	28.62 ± 0.46 ^a	28.36 ± 0.58 ^a	28.28 ± 0.35 ^a	29.00 ± 0.12 ^a	28.40 ± 0.11 ^a	28.96 ± 0.63 ^a
WBC (10 ³ /L)	22.29 ± 0.89 ^a	22.77 ± 0.75 ^a	22.37 ± 0.38 ^a	22.26 ± 1.56 ^a	22.25 ± 0.97 ^a	22.81 ± 0.90 ^a
Lymphocytes (%)	47.46 ± 1.89 ^a	47.09 ± 0.84 ^a	46.90 ± 2.89 ^a	46.22 ± 1.05 ^a	47.02 ± 2.03 ^a	50.72 ± 1.73 ^b
Eosinophils (%)	0.48 ± 0.02 ^a	0.48 ± 0.02 ^a	0.46 ± 0.02 ^a	0.48 ± 0.01 ^a	0.48 ± 0.01 ^a	0.47 ± 0.01 ^a
Neutrophils (%)	42.90 ± 1.17 ^a	41.68 ± 0.99 ^c	42.94 ± 2.09 ^a	44.26 ± 2.11 ^a	47.68 ± 2.13 ^b	48.11 ± 2.20 ^b

Data are means of six determinations ± SEM. Values with superscripts different from the control down each column for each parameter are significantly different (p < 0.05). D.g – *Dialium guineense* fruit pulp meal-based diet. Key :- D.g – *Dialium guineense* fruit pulp meal-based diet. RBC: Red Blood Cell, Hb - Haemoglobin , PCV – Packed Cell Volume , MCV – Mean Cell Volume , MCH – Mean Cell Haemoglobin , MCHC – Mean Cell Haemoglobin Concentration, WBC – White Blood Cell.

Dialium guineense fruit pulp meal-based diet at all the inclusion levels significantly increased (p < 0.05) the concentration of total cholesterol (TC) and high density lipoprotein cholesterol (HDL-C) compared with those maintained on basal diet (Table 4). For the total cholesterol concentration, rats fed on 5%, 10% and 20% *Dialium guineense* fruit pulp inclusion levels compared

favourably (p > 0.05) with each other. Likewise, rats fed on 20%, 30% and 40% inclusion levels also compared favourably (p > 0.05) with each other. For the HDL-C concentration, there was no significant difference (p > 0.05) in the concentration of this biomarker in the rats fed on 5%, 10% and 20% of the fruit pulp meal-based diet. In the same way, rats fed on

30% and 40% of the diets also compared favourably ($p > 0.05$) with each other. For low density lipoprotein concentration (LDL-C), triglyceride concentration (TG) and atherogenic index, the fruit pulp meal-based diet at

all inclusion levels (5% to 40%) resulted in significant decrease ($p < 0.05$) when compared with the control values but all the inclusion levels compared favourably ($p < 0.05$) with each other in the three parameters.

Table 4: Effect of *Dialium guineense* fruit pulp meal-based diet on serum lipid profile of rats

Page	Treatment	TC (mmol/L)	HDL-C (mmol/L)	LDL -C (mmol/L)	TG (mmol/L)	ATHEROGENIC INDEX
3175	Control	2.77 ± 0.05 ^a	1.94 ± 0.01 ^a	1.08 ± 0.12 ^a	1.78 ± 0.07 ^a	0.61 ± 0.02 ^a
	5% D.g	3.47 ± 0.06 ^b	2.58 ± 0.02 ^b	0.79 ± 0.03 ^b	0.98 ± 0.09 ^b	0.38 ± 0.03 ^b
	10% D.g	3.38 ± 0.14 ^b	2.72 ± 0.04 ^b	0.83 ± 0.04 ^b	0.89 ± 0.02 ^b	0.35 ± 0.04 ^b
	20% D.g	3.95 ± 0.17 ^{bc}	2.75 ± 0.06 ^b	0.71 ± 0.08 ^b	0.81 ± 0.08 ^b	0.28 ± 0.02 ^b
	30% D.g	4.19 ± 0.12 ^c	3.32 ± 0.11 ^c	0.69 ± 0.11 ^b	0.84 ± 0.12 ^b	0.27 ± 0.02 ^b
	40% D.g	4.33 ± 0.33 ^c	3.21 ± 0.21 ^c	0.71 ± 0.13 ^b	0.78 ± 0.02 ^b	0.26 ± 0.08 ^b

Data are means of six determinations ± SEM. Values with superscripts different from the control for each parameter are significantly different ($p < 0.05$). D.g – *Dialium guineense* fruit pulp meal-based diet. TC: Total Cholesterol, HDL-C: High Density Lipoprotein, LDL -C: Low Density lipoprotein Cholesterol, TG – Triglyceride.

The effect of *Dialium guineense* fruit pulp meal-based diet on the organ (liver and kidney) body weight ratios is presented in Table 5. The liver and kidney body weight ratios of the animals maintained on 5, 10, 20, 30

and 40% *Dialium guineense* fruit pulp meal-based diet (all the inclusion levels) were not significantly different ($p < 0.05$) from those maintained on the basal diet (control)

Table 5: Effect of *Dialium guineense* fruit pulp meal-based diet on the % liver- and kidney -body weight ratio of rats

Treatments	Organs (ratio in %)	
	Liver	Kidney
Control	4.02 ± 0.08 ^a	1.18 ± 0.11 ^a
5%	3.99 ± 0.14 ^a	1.23 ± 0.07 ^a
10%	4.06 ± 0.03 ^a	1.21 ± 0.09 ^a
20%	4.99 ± 0.19 ^a	1.19 ± 0.10 ^a
30%	4.04 ± 0.01 ^a	1.18 ± 0.13 ^a
40%	4.04 ± 0.06 ^a	1.17 ± 0.03 ^a

Data are means of six determinations ± SEM. Values with the same superscript a, across the same column for each organ are not significant different ($p > 0.05$). D.g – *Dialium guineense* fruit pulp meal-based diet.

Discussion

Red blood cells (RBC) (erythrocytes) serve as a carrier of haemoglobin. Haemoglobin has the physiological function of transporting oxygen to tissues of the animal for oxidation of ingested food so as to release energy for the other body functions as well as transport carbon dioxide out of the body of animals (Ugwuene, 2011; Omiyale *et al.*, 2012; Soetan *et al.*, 2013; Isaac *et al.*, 2013). Thus, the insignificant difference in the red blood cells as well as haemoglobin implies that there will be no reduction in the level of oxygen that would

be carried to the tissues as well as the level of carbon dioxide returned to the lungs (Ugwuene, 2011; Soetan *et al.*, 2013; Isaac *et al.*, 2013). This therefore indicates that there will be no traces of anaemia, dehydration, poor diet/nutrition, toxin infection, a malabsorption problem or any other ailment or disorder (Ganong, 2001). Packed Cell Volume (PCV) which is also known as haematocrit (Ht or Hct) or erythrocyte volume fraction (EVF), is the percentage (%) of red blood cells in blood (Purves *et al.*, 2003). According to Isaac *et al.* (2013), Packed Cell Volume is involved in the transport of oxygen and absorbed nutrients. As the PCV in the blood of animals fed on *Dialium guineense* fruit pulp meal based diet compared favourably with the control diet, this implies that number of Red Blood Cells and its transportation as well as plasma volume is not compromised. Mean Corpuscular Volume (MCV) measures the size of Red Blood Cells, Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) give the average weight and concentration of haemoglobin in the red blood cell respectively (Ganong, 2001). These three parameters depict blood level conditions (NseAbasi *et al.*, 2014). The insignificant difference in the MCV values suggests that the size of the red blood cells is normal because an abnormally small-sized red blood cell will either indicate a failure of the system to synthesize RBCs or lack of available haemoglobin to be incorporated into RBCs to complete the task of synthesis. Infact, low MCHC implies that a unit packed

RBCs contains less haemoglobin than normal and a high MCHC means that there is more haemoglobin in a unit of RBC (Ganong, 2001). Infact, these three parameters with the Packed Cell Volume and haemoglobin are major indices for evaluating circulatory erythrocytes and serve as useful indices of the bone marrow capacity to produce red blood cells as in mammals (Awodi *et al.*, 2005; Chineke *et al.*, 2006). Therefore, their insignificant differences in this study suggest that there was no alteration in their functions. Overall, the insignificant difference in the RBC (erythrocytes) and indices relating to it (Hb, PCV, MCV, MCH and MCHC) of the rats fed with the experimental diets (5% -40%) throughout the experimental period suggests that the fruit pulp of *Dialium guineense* did not interfere with the metabolism of the component of blood. This might be an indication that there was no destruction of matured RBC's and no changes in the rate of production of erythrocytes (Ganong, 2001). In like manner, there is an indication that the oxygen carrying capacity of the animals was not reduced.

White blood cells (WBC) or leucocyte counts are the total number of white blood cells (Robert, 1976). The main function of the white blood cells (WBC) and its differentials are to fight infection, defend the body by phagocytosis against invasion by foreign organisms and to produce or at least transport and distribute antibodies in immune responses (Ganong, 2001). There are a number of types (differentials) of leukocytes that are classified either as granulocytes or non-granulocytes. The granulocytes include neutrophils, eosinophils and basophils while the non-granulocytes include lymphocytes and monocytes (Ganong, 2001). The result obtained from the white blood cell count in this study implies that the experimental diet might not introduce any toxin/infection into the system of the animals as the integrity of resistance to diseases was not compromised (Soetan *et al.*, 2013). Although, there were slight variations in the lymphocytes and neutrophil counts in some groups and knowing the fact that these molecules are important in body defence, however, the overall result from white blood cell counts revealed that there was no compromise in the defence system of the body.

Lipids are fatty substances that are normally insoluble in an aqueous environment but are solubilised and transported in the plasma as water-soluble macromolecular complexes known as lipoproteins (Styrer, 1995). Measurement of major lipids can give useful information about the functioning of the heart and are particularly used to estimate increased risk of cardiovascular disease (Naik, 2012). Although, significant increase in total cholesterol can indicate a high risk of cardiovascular disease, the significant increase in the total cholesterol concentration of the all the groups fed on the fruit pulp meal based diet may not

be fully attributed to increased mobilization of free fatty acids from the peripheral fat depots since there was no concomitant increase in the low density lipoprotein (LDL) and triglyceride concentration in all the experimental groups and this might be justified by the increase in high density lipoprotein (HDL) cholesterol (Aguilar *et al.*, 2011). Increased LDL concentrations are associated with atherosclerosis, heart attack, stroke and cardiovascular diseases (Bordia and Verma, 1998; Cromwell and Otvos, 2004) and could enhance obesity. *Dialium guineense* fruit pulp meal based diet reduced LDL susceptibility to oxidation and possibly increased the resistance of plasma LDL to oxidation. This could therefore prevent obesity (Ijeh *et al.*, 2015). This might be due to the presence of flavonoids and other phenolic constituents in the fruit pulp (Oyegoke and Oladiji, 2014) as these free radicals scavenging secondary metabolites help prevent cardiovascular diseases by interfering with the oxidation of the very low density lipoproteins and low density lipoproteins, which are the chief engineers of atherosclerosis (Omodamiro and Nwankwo, 2013). Dietary incorporation of the fruit pulp of *Dialium guineense* also decreased the triglyceride concentrations, and could therefore improve hypertriglyceridemia and the associated obesity (Ijeh *et al.*, 2015). High Density lipoprotein are referred to as the "good cholesterol" since they remove cholesterol to the liver for excretion, therefore their significant increase will be appropriate for the increased total cholesterol and thus reduce the risk of coronary artery disease. Atherogenic index serves as a direct pointer to exposure to atherosclerosis which is a precursor of all cardiovascular diseases (Oloyede *et al.*, 2015). High atherogenic index may suggest that atherogenic plaques have been deposited on the walls of the arteries (Rosalki, 1967; Sartej *et al.*, 2012) and these may lead to partial or complete blockage to the flow of blood in the arteries exposing the individual to all forms of cardiovascular diseases (Oloyede *et al.*, 2015). In this study, since the computation for the atherogenic index reveals that there is no group in which the index was greater than 5 (Ng *et al.*, 1997), it can be inferred that the diet does not possess positive risk for atherogenesis. The fruit pulp probably plays atherogenic role through the inhibition of lipid oxidation and therefore could promote the reverse cholesterol transport pathway (Ijeh, 2015). This can be evidently supported from the result of high density lipoprotein concentration in this study. Oyegoke and Oladiji (2014) reported the presence of saponins and fibre in the fruit pulp of *Dialium guineense*. Saponins have been reported to reduce the uptake of cholesterol at the gut through intra-luminal physicochemical interaction (Price *et al.*, 1987). Likewise, fibre promotes a sense of satiety which helps to prevent overeating and weight gain (De Moura *et al.*, 2009).

Furthermore, dietary fibres slow down the rate of absorption of cholesterol in the gastrointestinal tract. Therefore, the serum lipid modulating property of this fruit pulp may therefore be attributable to its saponin and fibre content.

Analysis of organ weight in toxicology studies is an important endpoint for identification of potentially harmful effects of test compounds/ toxins (Bailey *et al.*, 2004). Organ body weight ratio is a marker of cell constriction and inflammation (Moore and Dalley, 1999). In some cases, the organ might shrink as a result of excess loss of fluid due to some abnormal conditions. At other times, the organ might swell abnormally suggesting excessive fluid retention, which might be due to osmotic pressure. The lack of significant change in the size of the liver and kidney relative to their entire weight of the animals suggest that the fruit pulp meal-based diet of *Dialium guineense* did not cause any inflammation or constriction in the cells of the organs investigated.

Conclusion

Overall, although there are some variations in the values of some haematological parameters, nevertheless, the present findings have shown that the fruit pulp of *Dialium guineense* is not likely to produce any severe toxicological effects on the haematological parameters, lipid profile and on organ weights when given at the experimental inclusions.

Conflict of Interest

The authors declare that there are no conflict of interest arising from this research.

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