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Safety evaluation of *Dialium guineense* fresh fruit pulp meal-based diet

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ABSTRACT: Toxicological investigation of plant species used as food/drug requires the examination of a wide array of assays including antioxidant parameters and tissues functional indices. This study investigated the safety of the fruit pulp of *Dialium guineense* in rats by assaying for liver and kidney antioxidant parameters as well as some selected functional indices. Thirty-six rats were assigned into 6 groups, A – F and maintained on basal diet, 5, 10, 20, 30 and 40% inclusion of *Dialium guineense* fruit pulp respectively for five weeks. The study revealed a non-significant difference ($p > 0.05$) in the antioxidant parameters except for catalase in which a significant decrease ($p < 0.05$) was observed at 30 and 40% in both tissues. For the aminotransferases, gamma glutamyl transferase, globulin, total and conjugated bilirubin as well as the five electrolytes (Na^+, K^+, HCO_3^{2-}, PO_4^{2-} ,Cl^-) assayed for, no significant difference ($p > 0.05$) was observed. However, rats maintained on the diet at all supplementation levels had a significant increase ($p < 0.05$) in the total protein, albumin and urea level except for creatinine. The insignificant effects in most parameters suggest that the pulp might not interfere with normal functioning of the biomolecules at the percentage inclusions.

Keywords: *Dialium guineense*, Antioxidant , Liver, Kidney, Inclusion

Introduction

Traditional medicine has a long history (Devi *et al*., 2016). It is the sum total of the knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures. This can be explicable or not and it is used in the maintenance of health, as well as in the prevention, diagnosis, improvement or treatment of physical and mental illnesses (Devi *et al*., 2016). Medicinal plants form an important component of the natural wealth of any country. The tropical rainforest of which Nigeria is a part has been described by Sofowora (1982) as a reservoir of phytomedicines. These plants have traditionally been used by Nigerians because, they are natural products, environmentally friendly, easily available, cheap and curative than many orthodox medicines (Murray, 1995). They are preferred because of the ills of conventional medications which include toxic effects on humans, resistance by man and animals, Non availability/ Non accessibility, High cost of most of the drugs (Egharavba and Ikhatua, 2008).

In line with the interaction of food in management of diseases, phytomedicines are also now preferred to be taken as food supplement instead of concoctions, decoctions etc. which goes in tandem with the use of orthodox drugs. However, the use of it in either ways as food or as drug still requires thorough safety evaluation because the toxicological level of most of them have not been established. Toxicology is the study of the adverse physicochemical effects of chemical, physical or biological agents on living organisms and the ecosystem. This includes the prevention and amelioration of such adverse effects.

The effects on organisms can occur at multiple levels, including the molecular and the organ levels. One of the most important assay required to determine the level of toxicity of a food or drug in the biological system is the assay of the antioxidant defense system. Reactive oxygen species are highly reactive, oxygen-containing molecules including the free radicals which cause biological damage known as oxidative stress to the tissues and cells of the body (Ridnour *et al*., 2005). These molecules when generated in the body system by introduction of toxins from the food or drug taken in are mopped by the antioxidant molecules through the antioxidant defense system therefore, the need to certify the integrity of this system.

Enzymatic defense system include the superoxide dismutase (SOD) and catalase (CAT) while the gluthathione is non-enzymatic. SOD catalyze the dismutation of superoxide anions (O_2-) generated in the gastrointestinal tract to hydrogen peroxide (H_2O_2) and oxygen (O_2) rendering the potentially harmful superoxide anion less hazardous thereby protecting the mucosal and the epithelial cells. Catalase catalyzes the reduction of H_2O_2 generated as a result of oxidative stress in the GI tract to water and oxygen using either an iron or manganese cofactor (Chelikani *et al*., 2004). Gluthathione (GSH) is a major thiol based non-enzymic antioxidant in living organism, which performs a key role in co-ordinating the innate antioxidant defence mechanisms. GSH acts as ROS scavenger in the stomach and duodenum (Koevary, 2012).

Another in the series antioxidant defense system assay is the lipid peroxidation, which is a process induced by free radicals leading to oxidative deterioration of polyunsaturated fatty acids. The lipid peroxidation product, malondialdehyde, is commonly used as a measure of the oxidative stress in cells. Lipid peroxidation, being a free radical reaction, occurs when the hydroxyl radicals, possibly oxygen, react with the unsaturated lipids of the bio-membranes, resulting in the generation of lipid peroxide radicals (ROO^-), lipid hydroperoxide (ROOH) and fragmentation products such as malondialdehyde. This aldehyde is a highly toxic molecule and is a marker of lipid peroxidation. Its interaction with the DNA and proteins has often been referred to as potentially mutagenic and atherogenic (Marnett, 1999). Also of toxicological relevance are the aminotransferases and the gamma glutamyl transferase enzymes. Alanine aminotransferase (ALT) catalyzes the transamination reaction between L-alanine and α-ketoglutarate (George *et al.*, 1994), to form pyruvate and glutamate while aspartate aminotransferase (AST) catalyses the transamination reaction between L-aspartate and α-ketoglutarate to form glutamate and oxaloacetate at optimum pH of 7.4.

ALT is less abundant than AST in human tissues (Wilkinson, 1963), it is native to the cytosol, though small amount have been found in the mitochondria of liver cells and also in the heart muscles (Wilkinson, 1963; Bhargavan and Sreenivasan, 1965). AST is widely distributed in animal tissues with a relatively high concentration in the heart muscle (Bhargava and Screenivasan, 1965).

The liver, kidney, pancreas, lung and spleen also contain considerable quantities of the enzyme (Miller and Luthrade, 1990). It is found both in the cytosol and in the mitochondria of the cells (Boyd, 1960). Both enzymes are very important in the diagnosis of liver and kidney diseases caused by drug toxicity or infection (Nelson and Cox, 2005). Another in this series is the gamma glutamyl transferase enzyme which is a membrane localized enzyme. It catalyzes the transfer of amino acid from one peptide to another amino acid or peptide (i.e. it acts as amino acid transferase) (Burtis and Ashwood, 2001). Gamma glutamyl transferase plays a major role in glutathione metabolism and reabsorption of amino acids from the glomerular filterate and from intestinal lumen (Kaplan and Pesce, 1996). The enzyme is found in a number of tissues. It occurs mainly in the cells of the liver, kidney, pancreas and prostate. It is

also present in the plasma membrane of renal tubular cells, endoplasmic reticulum of the hepatocytes (Murray *et al.,* 2000) and seminal vesicles (Kohdaira *et al.,* 1986).

Serum levels of gamma glutamyl transferase are commonly elevated in response to many drugs and toxins (Ruppin *et al.,* 1982). In the same vein, liver and kidney function tests are also very important guage in assessing the function of these organs as well as determining if there are signs of toxicity. The concentration of proteins, bilirubin and albumin in the serum can be used to ascertain the state of the liver and different types of liver damage while the kidney function parameters include urea, creatinine, uric acid and serum electrolytes like K^+, Na^+, PO_4^{2-}, HCO_3^{2-}, Cl^- (Yakubu *et al*., 2003).

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). *D. guineense* is used as chewing stick (indigenous tooth brush) among Nigerian populace (Akinpelu *et al*., 2011), also the stem bark as well as the leaves are also used as folklore remedies for the treatment of infections such as diarrhoea, severe cough, bronchitis, wound, stomach aches, malaria fever, jaundice, ulcer and haemorrhoids (Bero *et al*., 2009). The fruit pulp of the plant is claimed locally to have anti-ulcerogenic potential (Aiyeloja and Bello, 2006) and this have been scientifically proven by Oyegoke and Oladiji (2014). Some of the other scientifically validated activities of the plant leaves, stem bark and fruit pulp include its analgesic and antibacterial activities, antioxidant and antimicrobial activities (Gideon *et al*., 2013).

The upsurge in the use of this plant in the flora as herbal remedy for anti-ulcer efficacy and others necessitate thorough scientific investigation in order to provide information on its safety and toxicity risk. In the same vein, there is dearth of information on this plant especially the fruit pulp on its toxicological implications in open scientific literature; therefore the need to provide information to fill this lacuna. Therefore, the main objective of the study was to investigate the safety /toxicity risk of *Dialium guineense* fruit pulp meal-based diet while the specific objectives evaluate the safety of the diet in rats as it relates to antioxidant enzymes, protein and non- antioxidant enzymes as well as liver and kidney function indices**.**

Materials and Methods

Plant Material

The fruit pulp of *Dialium guineense* was purchased from Dawanu Market in Kano City, Nigeria. It 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 novergicus*) 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.

Ingredients for Feed formulation and their sources

Yellow maize seeds, cellulose (corn cob) and soybean were purchased from Oja Oba Market, Ilorin, Nigeria. Sunola Refined soybean oil is a product of Kewalrams Nigeria Limited while the vitamin/mineral mix, lysine and D-methionine were purchased from Rofiat Feeds Nigeria Limited.

Chemicals and Assay Kits

The chemicals and assay kits used in this study were of the finest qualities commercially available. Albumin, bilirubin, urea, creatinine, alanine aminotransferase, aspartate aminotransferase and gamma glutamyl transferase were purchased from Randox Laboratories Limited, United Kingdom. Other reagents were purchased from British Drug Houses (BDH), Poole, England.

Preparation of Plant Material

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^{o}C and pulverized in an electric blender (Super Master Electrical Appliance Industries Co., Kyoto, Japan) and then stored for later use.

Composition of Diet

The Composition of diet is presented in Table 1.

Table 1: Composition of the diet (g/kg)

Ingredient	Control (Basal diet)	*Dialium guineense* fruit pulp meal-based diet				
		5%	10%	20%	30%	40%
Corn Starch	506	480.70	455.40	404.80	354.20	303.60
D. guineense 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 Mix	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 D_3, 800,000 i.u.; Tocopherols, 400 i.u; Vitamin K_3 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.

Animal Grouping

36 rats were acclimatized for one week. They were kept in well-ventilated house conditions Temperature: 22 ±3^{0}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 of acclimatization, the animals were grouped into six as follows:

A – Control group fed on Control (Basal) Diet
B - Group fed on 5% *Dialium guineense* fruit pulp meal-based diet
C - Group fed on 10% *Dialium guineense* fruit pulp meal-based diet
D - Group fed on 20% *Dialium guineense* fruit pulp meal-based diet
E - Group fed on 30% *Dialium guineense* fruit pulp meal-based diet
F - Group fed on 40% *Dialium guineense* fruit pulp meal-based diet

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

Preparation of serum and tissue homogenates

The procedures described by Akanji and Ngaha (1989) were used to collect the blood while the tissues (Liver and kidney) homogenates as well as serum were prepared according to the procedure described by Yakubu *et al* (2003).

Procedures for determination of various biochemical parameters for safety evaluation study
Determination of the activities of antioxidant enzymes and protein concentration

Superoxide dismutase activity was determined by the method described by Mistra and Fridovich (1972). Catalase was assayed according to the procedure described by Beers and Sizer (1952). Reduced

glutathione concentration was determined using the procedure of Ellman (1959) while the concentration of malondialdehyde (MDA) was determined using the method of Buege and Aust (1978).

Determination of the activities of the Aminotransferases and Gamma glutamyl transferases

The procedure described by Reitman and Frankel (1957) was used to assay for the activity of aspartate aminotransferase and alanine aminotransferase while the method described by Szasz (1969) was used to assay for the activity of gamma glutamyl transferase.

Determination of liver and kidney function tests

The protein concentration in the serum of the animals was assayed, using Biuret reagent as described by Gornall *et al* (1949) while the procedure described by Doumas *et al* (1971) was used for the determination of albumin in the serum of the animals. The determination of serum globulin content was done using the method described by Tietz (1995) by subtracting the concentration of serum albumin from the total serum protein content. Bilirubin was determined using the method described by Sherlock (1951). The method used for the determination of urea in the serum was that described by Veniamin and Vakirtzi (1970). Serum creatinine was carried out using the procedure described by Bartels and Bohmer (1972). For the determination of the electrolytes, sodium ion, potassium ion, bicarbonate ion, phosphate ion and chloride ion were determined by the method of Tietz (1995), Tietz (1990), Tietz (1995), Fiske and Subbarrow (1925) and Skeggs and Hochstrasser (1964) respectively.

Statistical Analysis

Data was expressed as mean values ± SEM of six replicates. All results were statistically analyzed using one-way ANOVA and Duncan's Multiple Range Test. Differences between group means were considered significant at $P < 0.05$.

Results

Effect of *Dialium guineense* fruit pulp meal-based diet on the activities of liver and kidney antioxidant enzymes and protein concentration

The effect of *Dialium guineense* fruit pulp meal-based diet on the liver and kidney superoxide dismutase and catalase activities, reduced glutathione and malondialdehyde concentrations of rats is shown in Table 2. Feeding of rats on *Dialium guineense* fruit pulp meal-based diet at all inclusion levels did not significantly alter ($p > 0.05$) the liver and kidney superoxide dismutase activity, reduced glutathione and malondialdehyde concentrations when compared with the control values. However, for the catalase activity in the liver and kidney, there was a significant reduction ($p < 0.05$) in rats maintained on 30 and 40% of the diet whereas the activity of the enzyme was not altered ($p > 0.05$) in rats maintained on 5%, 10% and 20% of the diet when compared with the control values.

Effect of *Dialium guineense* fruit pulp meal-based diet on the liver and kidney aminotransferases and liver gamma glutamyl transferase activities of rats

The effect of *Dialium guineense* fruit pulp meal-based diet on the liver and kidney aminotransferases and liver gamma glutamyl transferase activities of rats is presented in Tables 3 and 4 respectively. *Dialium guineense* fruit pulp meal-based diet at 5% to 40% inclusion levels did not produce any significant change ($p > 0.05$) in the activities of alanine aminotransferase and aspartate aminotransferase in the liver and kidney as well as liver gamma glutamyl transferase when compared with the animals maintained on the basal (control) diet (Tables 3 and 4). Similarly, there was no significant difference ($p > 0.05$) in the activities of the three enzymes in the serum of rats when compared with the control values.

Table 2: Effect of *Dialium guineense* fruit pulp meal-based diet on the activities of liver and kidney antioxidant enzymes and protein concentration

Treatment Groups	Superoxide Dismutase		Catalase		Reduced Glutathione		Malondialdehyde	
	Liver	Kidney	Liver	Kidney	Liver	Kidney	Liver	Kidney
Control	39.62 ± 0.47[a]	25.40 ± 1.01[a]	26.97 ± 1.08[a]	17.28 ± 0.99[a]	18.45 ± 1.44[a]	11.56 ± 1.02[a]	0.22 ± 0.02[a]	0.25 ± 0.02[a]
5% D.g	38.84 ± 0.67[a]	24.92 ± 0.95[a]	26.78 ± 1.96[a]	17.02 ± 0.56[a]	18.58 ± 1.23[a]	11.77 ± 0.78[a]	0.26 ± 0.01[a]	0.26 ± 0.01[a]
10% D.g	39.13 ± 0.98[a]	25.21 ± 1.03[a]	26.88 ± 1.01[a]	16.98 ± 1.02[a]	18.49 ± 0.98[a]	11.98 ± 0.89[a]	0.24 ± 0.03[a]	0.26 ± 0.04[a]
20% D.g	38.79 ± 0.68[a]	24.98 ± 1.44[a]	26.62 ± 0.98[a]	16.99 ± 0.78[a]	18.02 ± 1.06[a]	11.03 ± 0.85[a]	0.24 ± 0.01[a]	0.25 ± 0.03[a]
30% D.g	40.01 ± 0.02[a]	24.98 ± 0.89[a]	24.43 ± 0.46[b]	15.44 ± 0.56[b]	18.39 ± 0.95[a]	11.40 ± 0.56[a]	0.21 ± 0.03[a]	0.24 ± 0.01[a]
40% D.g	39.12 ± 0.78[a]	25.78 ± 0.40[a]	24.41 ± 1.07[b]	15.56 ± 0.78[b]	18.24 ± 1.41[a]	11.05 ± 1.02[a]	0.20 ± 0.04[a]	0.25 ± 0.07[a]

Data are means of six determinations ± SEM. Values with superscripts different from the control for each parameter down each column are significantly different ($p < 0.05$). *D.g – Dialium guineense* fruit pulp meal-based diet.

Table 3: Effect of *Dialium guineense* fruit pulp meal-based diet on the liver, kidney and serum aminotransferase activities of rats

Treatment Groups	Alanine aminotransferase (U/L)			Aspartate aminotransferase (U/L)		
	Liver	**Kidney**	**Serum**	**Liver**	**Kidney**	**Serum**
Control	58.51 ± 2.01[a]	28.65 ± 1.02[a]	1.52 ± 0.09[a]	202.15 ± 4.32[a]	131.40 ± 3.64[a]	1.03 ± 0.04[a]
5% D.g	57.29 ± 1.09[a]	27.95 ± 0.98[a]	1.50 ± 0.05[a]	203.01 ± 3.12[a]	132.10 ± 2.46[a]	1.05 ± 0.03[a]
10% D.g	57.32 ± 0.56[a]	28.45 ± 1.03[a]	1.46 ± 0.03[a]	203.43 ± 0.14[a]	132.37 ± 1.69[a]	1.07 ± 0.06[a]
20% D.g	57.44 ± 1.08[a]	27.75 ± 1.32[a]	1.56 ± 0.04[a]	202.14 ± 2.11[a]	131.65 ± 1.30[a]	1.06 ± 0.08[a]
30% D.g	58.03 ± 0.23[a]	28.52 ± 0.98[a]	1.50 ± 0.07[a]	203.15 ± 2.13[a]	132.16 ± 3.59[a]	1.01 ± 0.09[a]
40% D.g	58.62 ± 1.02[a]	28.44 ± 1.32[a]	1.54 ± 0.02[a]	203.98 ± 2.14[a]	131.38 ± 2.59[a]	1.05 ± 0.04[a]

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

Table 4: Effect of *Dialium guineense* fruit pulp meal-based diet on liver gamma glutamyl transferase activity of rats

	Gamma glutamyl transferase (U/L)	
Treatments	**Liver**	**Serum**
Control	256.12 ± 5.12[a]	3.95 ± 0.12[a]
5% D.g.	255.45 ± 4.98[a]	4.02 ± 0.65[a]
10% D.g.	254.16 ± 3.87[a]	4.34 ± 0.18[a]
20% D.g.	254.47 ± 2.98[a]	4.04 ± 0.68[a]
30% D.g.	253.11 ± 3.25[a]	3.87 ± 0.45[a]
40% D.g.	253.23 ± 2.29[a]	3.98 ± 0.67[a]

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

Effect of *Dialium guineense* fruit pulp meal-based diet on liver and kidney function indices of rats

The effect of *Dialium guineense* fruit pulp meal-based diet on liver function indices is shown in Table 5. *Dialium guineense* fruit pulp meal-based diet significantly increased ($p < 0.05$) the serum total protein and serum total albumin concentrations in rats at all inclusion levels (5% - 40%) when compared with the control values. For the protein and albumin concentration, there was also a significant decrease ($p < 0.05$) in rats fed on 5%, 10% and 20% of the diet when compared with those fed on 30% and 40% of the diet. However, the rats maintained on 30% and 40% supplementation compared favourably ($p > 0.05$) with each other. There was no significant difference ($p > 0.05$) in the serum globulin, total bilirubin and conjugated bilirubin levels of rats fed on the diet at all inclusion levels when compared with the control values. The effect of *Dialium guineense* fruit pulp meal-based diet on kidney function indices of rats is presented in Table 6. Feeding of rats with *Dialium guineense* fruit pulp meal-based diet significantly increased ($p < 0.05$) the urea and creatinine concentration in the serum of rats when compared with the control values. However, for the creatinine concentration, there was a significant decrease ($p < 0.05$) in rats fed on 20%, 30% and 40% of the diet was compared with those fed on 5% and 10% of the diet. For

all the electrolytes, Na^+, K^+, HCO_3^{2-}, PO_4^{2-} and Cl^-, there was no significant difference ($p > 0.05$) in all the rats fed on the diet when compared with the control value.

Table 5: Effect of *Dialium guineense* fruit pulp meal-based diet on liver function indices of rats

	Treatment Groups					
Parameters	**Control**	**5% D.g**	**10% D.g**	**20% D.g**	**30% D.g**	**40% D.g**
Total protein (mg/ml)	43.57 ± 2.11^a	45.29 ± 1.04^b	45.32 ± 2.45^b	46.16 ± 1.07^c	47.69 ± 1.45^d	47.36 ± 1.04^d
Albumin (g/l)	25.08 ± 1.02^a	27.42 ± 0.45^b	27.11 ± 1.45^b	27.93 ± 1.39^b	28.83 ± 0.03^c	28.30 ± 1.00^c
Globulin (g/l)	18.13 ± 0.34^a	18.21 ± 0.35^a	18.89 ± 0.44^a	18.84 ± 1.14^a	18.34 ± 1.19^a	18.51 ± 1.04^a
Total bilirubin (µmole/l)	12.45 ± 1.06^a	12.29 ± 1.11^a	12.31 ± 0.08^a	12.37 ± 1.01^a	12.39 ± 0.98^a	12.38 ± 0.69^a
Conjugated bilirubin (µmole/l)	5.12 ± 0.23^a	4.99 ± 0.78^a	5.01 ± 0.92^a	5.08 ± 0.29^a	5.03 ± 0.61^a	5.08 ± 0.56^a

Data are means of six determinations ± SEM. Values with superscripts different from the control down each column for each parameter are significantly different ($p < 0.05$). Key :- D.g – *Dialium guineense* fruit pulp meal-based diet.

Table 6: Effect of *Dialium guineense* fruit pulp meal-based diet on kidney function indices of rats

	Treatment Groups					
Parameters	**Control**	**5% D.g**	**10% D.g**	**20% D.g**	**30% D.g**	**40% D.g**
Urea (mmol/l)	28.62 ± 1.03^a	31.43 ± 2.01^b	32.04 ± 1.07^b	32.92 ± 1.05^b	33.56 ± 0.43^b	32.99 ± 1.01^b
Creatinine (µmole/l)	90.12 ± 2.16^a	85.43 ± 1.01^b	85.62 ± 0.32^b	83.32 ± 1.41^c	83.21 ± 0.33^c	82.82 ± 0.56^c
Na+	22.14 ± 1.12^a	22.22 ± 1.21^a	21.61 ± 0.22^a	22.11 ± 1.06^a	21.83 ± 1.45^a	21.78 ± 1.98^a
K+	2.13 ± 0.23^a	2.49 ± 0.33^a	2.74 ± 0.45^a	2.18 ± 0.09^a	2.08 ± 0.16^a	2.14 ± 0.08^a
HCO3-	3.89 ± 0.68^a	3.88 ± 0.84^a	3.79 ± 0.45^a	3.90 ± 0.11^a	3.92 ± 10.68^a	3.52 ± 0.23^a
PO43-	0.78 ± 0.03^a	0.68 ± 0.05^a	0.69 ± 0.01^a	0.72 ± 0.06^a	0.77 ± 0.04^a	0.71 ± 0.06^a
Cl-	28.68 ± 1.69^a	28.22 ± 1.68^a	27.45 ± 0.98^a	28.42 ± 1.69^a	28.44 ± 0.98^a	27.46 ± 2.11^a

Data are means of six determinations ± SEM. Values with superscripts different from the control down each column for each parameter are significantly different ($p < 0.05$). Key :- D.g – *Dialium guineense* fruit pulp meal-based diet.

Discussion

Dialium guineense fruit pulp has been scientifically investigated and proven for various nutritional and therapeutic uses. However, a holistic investigation of its possible toxic effects is needed for proper pharmaceutical and nutritional use. Evaluation of antioxidant parameters as well as liver and kidney function indices are some of the pointers that have been used to predict the effects of foreign compounds (such as plant products) on body systemic functions (Anderson, 2001).

Free radicals scavenging enzymes and protein such as superoxide dismutase (SOD) and catalase (CAT), reduced glutathione are known to be the first line of cellular defense against oxidative damage, disposing O_2 and H_2O_2 before their interaction to form the more harmful hydroxyl ($OH^{\cdot}$) radical (Lil *et al*., 1988). SOD is an important defense enzyme that catalyzes the dismutation of superoxide anions into O_2 and H_2O_2 (Bannister and Bannister, 1987). CAT is a hemeprotein that catalyzes the reduction of H_2O_2 to H_2O and O_2 and protects the tissue from highly reactive oxygen free radicals and hydroxyl radicals (Zamocky and Koller, 1999).

In the present study, the activities of SOD and CAT in liver and kidney were not significantly affected which suggests that there was apparently a reduced formation of superoxide anions which might

have inactivated these enzymes and decreased their activities. Reduced glutathione is one of the most abundant non-enzymatic antioxidant bio-molecules present in the tissues (Meister, 1984). Its functions include removal of free oxygen species such as H_2O_2, superoxide anions and alkoxy radicals, maintenance of membrane protein thiols and to act as a substrate for GSH-Px (Townsend, 2003).

The absence of significant effect on the reduced glutathione concentration of rats maintained on the fruit pulp meal-based diet also supports the findings obtained earlier from superoxide dismutase and catalase activities and this might be due to the non-interference of the fruit pulp with the synthesis of this biomolecule or by the presence of various bioactive molecueles like tannins and/ or flavonoids in the pulp (Oyegoke and Oladiji, 2014) which might enhance the activity of this biomolecule. The absence of significant effect in both the liver and kidney malondialdehyde levels corroborate with earlier results on the antioxidant enzymes and protein and suggest that there was no peroxidation that might lead to tissue damage. This implies that the antioxidant defense mechanisms might still be intact. Aminotransferase which include alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are enzymes located in the cytosol and mitochondria where they are involved in the transfer of amino group from α-amino to α-keto acids. They are also involved in the biochemical regulation of intracellular amino acid pool (Chapatwala *et al.,* 1982). These aminotransferases belong to the plasma non-functional enzymes which are normally localized within the cells of liver, heart, kidney and muscles. Their presence in serum may give information on tissue injury or organ dysfunction (Wells *et al.,* 1986).

Tissue and serum levels of ALT and AST can be used to assess the toxic impact of chemical compound including plant derived substances. The absence of effect on the activities of both enzymes in the organs and serum suggests that the function of the organs was not compromised by the diet (Wells *et al.,* 1986). γ-Glutamyl transferase (GGT) is the most sensitive enzymatic indicator of hepatobiliary disease (Mayne, 1998). It is a membrane localized enzyme that plays a major role in the glutathione metabolism and reabsorption of amino acid from the glomerular filterate and intestinal lumen (Kaplan and Pesce, 1996). The absence of significant effect in the activities of GGT in the liver and serum of rats fed on the fruit pulp meal-based diet may suggest that the activities of the liver had not been compromised.

The concentrations of albumin, globulin and bilirubin in the serum of the animals can indicate the secretory and synthetic functioning of the liver and can be used to ascertain types of liver damage (Yakubu *et al.,* 2003). Albumin is the major protein present within the blood and represents a reliable test to assess the degree of liver damage in animals. Albumin which is manufactured by the liver, is a major protein that circulates in the blood stream (Tietz, 1986). The increase in albumin concentration of rats fed on fruit pulp meal-based diet is an indication of dehydration (Naganna, 1989). This might also be due to increased rate of hepatic synthesis of albumin without proportionate increase in the rate of its elimination (Yakubu *et al.,* 2003). Globulins are a larger protein than albumins and are important for its immunological responses (Tietz, 1986). They are also an heterogenous complex mixture of protein molecule whose role is to regulate osmotic pressure (homeostatis) (Ganong, 2001). The absence of significant effect on serum globulin concentration of rats maintained on fruit pulp meal-based diet of *Dialium guineense* suggest that the rate of transportation of nutrients, defence, coagulation processes, buffering capacity of the blood and haemaostasis was not altered (Ganong, 2001).

Bilirubin is the major breakdown product that results from the destruction of old red blood cells. It is removed from the blood by the liver, hence it is a good indication of the function of liver (Murray *et al*., 2000). Bilirubin concentration is elevated in the blood either by increased production of bilirubin or decreased liver uptake (as a result of liver disease).

The findings in the present study showed that there was no significant effect on the total and conjugated bilirubin of rats fed with the fruit pulp meal-based diet of *Dialium guineense* at all supplementation (5% to 40%) when compared to the control. This observation might suggest that the experimental diet had no adverse effect on the liver (Chebeseborough, 1992). Renal function indices such as serum electrolytes (sodium, potassium, phosphate, chloride and bicarbonate), urea and creatinine can be used to evaluate the functional capacity of the nephrons of animals at the glomerular and tubular levels (Yakubu *et al.,* 2003).

Although, creatinine, urea and uric acids are major catabolic products of muscle, protein and purine metabolism, creatinine is regarded as the most endogenous marker in the diagnosis and treatment of kidney disease and its clearance is estimated as an indication of glomerular filtration rate (Chawla, 1999). The significant reduction in the concentration of creatinine at all inclusion levels of *Dialium guineense* fruit pulp meal-based diet when compared with the control group may be an indication of glomerular or tubular dysfunction (Saad *et al*., 2006).

Urea plays a key role in the countercurrent exchange system of the nephrons, which allows for reabsorption of water and critical ions. The significant increase in the serum urea concentration of all the groups of animals fed on the fruit pulp meal-based diet may be an indication of glomerular dysfunction of the nephron (Chawla, 1999). Electrolytes are present in the body and the balance of the electrolytes in the body is essential for normal function of cells and organs. The concentrations of electrolytes in the serum of animals could give an insight into the effect of chemical compound including plant/food substances on tubular or glomerular part of the kidney (Ashafa *et al.,* 2009). The absence of significant effect of the levels of all the electrolytes investigated in the animals fed on the diet at the various inclusion levels implies that the fruit pulp might not interfere with the metabolism of these electrolytes.

Conclusion

The absence of significant effects on the activity of most enzymes and concentrations of metabolites investigated for the safety evaluation studies suggest that the fruit pulp supplementation might not interfere with the normal functioning of the enzymes and metabolites. This implies that the fruit pulp may be safe for consumption at the percentage inclusion evaluated.

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