Food ©2010 Global Science Books

Preliminary Toxicological Evaluation and Effect of the Seed Oil of *Hura crepitans* and *Blighia unijugata bak* on the Lipid Profile of Rats

Rotimi Ayodele Oderinde • Adewale Adewuyi* • Ibironke Adetolu Ajayi

Department of Chemistry, University of Ibadan, Ibadan, Oyo State, Nigeria

Corresponding author: * walexy62@yahoo.com

ABSTRACT

A preliminary investigation of the possible toxicity effect of the seed oil of *Hura crepitans* and *Blighia unijugata* Bak. was carried out on histopathology, some haematological and biochemical parameters of albino rats. The rats were divided into six groups of five rats each (A, B, C and D were fed with 5% of the test oils, E served as control without oil and F was fed with 5% of known edible oil) and fed for 12 weeks. The phytochemical screening of these oils reveals the presence of glycosides, steroids and flavonoids. The highest body weight gain was found in the group fed with oil from *B. unijugata*. There was a general difference in the weight of the kidney of all the test groups when compared with that of the control. No death or clinical sign of toxicity was observed in any of the groups. The study shows varying degree of vascular stress characterized by congestive lesions and also the possibility of cellular lipotoxicity based on the alteration of the lipid profile.

Keywords: cholesterol, haematology, histopathology, phytochemical screening

INTRODUCTION

High dietary fat is a risk factor for hypercholesterolemia, atherosclerosis, cardiovascular diseases and obesity. This can affect the absorption of cholesterol synthesis, synthesis of biliary acids, number and activity of low-density lipoprotein (LDL) receptors (Ros 2000). It has been shown that not only the amount of fat consumed but also the type of fatty acid influences the serum cholesterol (Yaqoob *et al*. 1995). The ingestion of polyunsaturated fatty acids present in vegetable oils is inversely related to the incidence of heart diseases by decreasing cholesterol and triacylglycerol plasmatic levels (Botham *et al*. 2003). It leads to the reduction of cholesterol levels, which is mainly LDL.

Hyperlipidemia or high levels of serum triacylglycerol (TG) and cholesterol is a risk factor for premature atherosclerosis, which is the underlying cause of heart attack, stroke and peripheral vascular diseases. These diseases can be viewed as a form of chronic inflammation that is induced and perturbed by lipid accumulation (Yang *et al*. 2006).

Blighia unijugata Bak. and *Hura crepitans* (HC) are trees planted as shade trees. HC, commonly known as the sand box tree, is about 25 m tall with a very spiny trunk and branches. The oil is used as a purgative; the wood is yellowish with a silky luster, light in weight and of good strength (Burkill 1994). *B. unijugata* is attractive especially when in fruit, which are red or pinkish yellow. The wood is used for buildings; it is also recognized for its sedative and analgesic properties in the treatment of rheumatism (Burkill 2000).

The aim of the present study was to investigate the preliminary toxicity, and effect of cholesterol and haematological indices of the seed oils of HC and *B. unijugata* on rats.

MATERIALS AND METHODS

Plant material

The seeds of both species were collected at the premises of University of Ibadan, Ibadan, Oyo State, Nigeria. They were identified at the Herbarium Unit, Botany Department, University of Ibadan.

Preparation and extraction

The seeds of *B. unijugata* were manually separated from the aril (AL) and cracked in order to remove the kernel (KL). These seeds including those of HC were ground separately in a laboratory mill.

Oil was extracted from KL, AL, and a KL/AL mixture in 1:1 ratio (KAL) of both plants' seeds using a soxhlet extractor with petroleum ether (40-60°) for 10 h (Ajayi *et al*. 2004).

Phytochemical analysis

The phytochemical analysis of the oils was performed by testing for tannins, saponin, phlobatannins, simple sugars, cardiac glycosides, steroids and flavonoids using standard procedures (Odebiyi and Sofowora 1978).

Experimental design and administration of oil

The experimental animals were divided into six groups of five rats each. Rats in groups A to D were fed with 5% (v/w) KL, AL, KAL and HC respectively. Group E was fed with 5% groundnut oil (a known edible oil), while group F was fed with feed without oil as control. The animals were properly housed at constant temperature and humidity and given free access to food and water. Average feed intake and body weight were recorded weekly for a period of 12 weeks (Ajayi *et al*. 2006).

Received: 3 July, 2008. Accepted: 3 September, 2010.

Research Note

Collection of blood samples for haematology and biochemical analysis

For the haematological parameters blood samples were collected into clean dry vials containing ethylenediamine tetracetate (EDTA) as an anticoagulant. For serum, blood was collected in dry clean vials without anticoagulant and allowed to clot. Clotted blood was then centrifuged for 10 min at 3000 rpm and separated sera stored at –20°C until analysis (Lee *et al*. 2007).

Sample collection for histopathology

Small pieces (2-3 mm) of tissues were cut from different organs namely; heart, kidney, liver, intestine and spleen, examined at necropsy and immediately transferred into 10% neutral buffered formalin (Skim *et al*. 1998).

Histopathological analysis

Fixed samples were trimmed and processed for paraffin-embedding sections (5-7 μm), which were placed on clean glass slides. After dewaxing and rehydration through a decreasing ethanol series, the sections were stained with haematoxylin and eosin and examined microscopically (Skim *et al*. 1998).

Haematological methods

Haemoglobin concentration (Hb), packed cell volume (PCV), red blood cell count (RBC), white blood cell count (WBC), platelet, mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), plasma protein, fibrogen, segmented neutrophils, band neutrophils, lymphocytes, monocytes and eosinophils were determined by standard methods (Schalm *et al*. 1975).

Biochemical method

Sera samples were analyzed for glucose (GLU), total bilirubin (T.Bil), alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), globulin (Glob), total protein (TP), albumin (ALB), creatinine (CREA), total cholesterol (T.chol), triglyceride (TG), high density lipoprotein (HDL) and low density lipoprotein (LDL) applying colorimetric procedures (Corinna *et al*. 2006). Values were read using a spectrophotometer (Corning colorimeter 292, Corning Ltd, Halstead, Essex, UK).

Statistical analysis

Data were analyzed by one-way ANOVA ($P < 0.05$) and least significant differences between treated means were determined by Duncan's multiple range test ($P < 0.05$) (Duncan 1955).

RESULTS AND DISCUSSION

Phytochemical screening

Table 1 shows the result of the phytochemical screening of the test oils. Glycosides and steroids were present in all the oils. Flavonoids were only found in AR, KAL and KL. The absence of tannins and saponins, which are antinutritional agents, shows some level of consumption safety of these oils. The presence of steroids also indicates that these oils are of seed source.

Table 1 Phytochemical screening of the oils.

Test	AR	KL	KAL	HC
Glycoside	+	+	+	+
Reducing sugar	-	-	-	-
Flavonoid	+	+	+	-
Steroid	+	+	+	+
Phlobatannins	-	-	-	-
Anthraquinone	-	-	-	-
Tannin	-	-	-	-
Saponin	-	-	-	-

Results from five determinations
AR = aril; HC = *Hura crepitans*; KAL = aril + kernel (1:1); KL = kernel

Table 2 Feed intake, body weight gain and feed efficiency of rats.

Group	Body weight gain (g)	Feed intake (g)	Feed efficiency*
A	248.00 ± 4.80 a	628.11 ± 6.30 a	0.395 ± 0.05 a
B	205.30 ± 2.30 b	604.21 ± 4.80 b	0.339 ± 0.02 b
C	213.10 ± 4.20 c	613.33 ± 4.10 c	0.347 ± 0.01 c
D	146.51 ± 2.20 d	580.20 ± 3.60 d	0.253 ± 0.02 d
E	100.07 ± 3.40 e	580.20 ± 3.60 e	0.216 ± 0.04 e
F	138.00 ± 4.30 f	515.00 ± 5.40 f	0.268 ± 0.02 f

Means ± standard deviation of five determinations. Data in a column with different letters are statistically different ($P \leq 0.05$) according to DMRT.

*Food efficiency = $\frac{\text{Body weight gain}}{\text{Feed intake}}$

Effect of oil on growth

The control group had the lowest average body weight gain (110.07 ± 3.40) and food efficiency (0.216 ± 0.04) over the 12 weeks' period as shown in **Table 2**. There was a significant difference in the body weight gain, feed intake and feed efficiency among all the studied groups. Group A fed with oil from the aril had the highest feed intake and the best feed efficiency of all the groups. The high body weight gain observed in groups A, B and C may be due to the deposition of fat in the body system of the rats. The body weight of the rats in group F fed with known edible oil (groundnut oil) is lower than others and this may be due to the ability of these rats to excrete the fat deposits on time or its conversion to energy.

Effect of oils on organs

A significant increase in weight was observed in the liver on comparing those of the control with others except for those in group F fed with known edible oil. This increase in weight may also be as a result of the inability of the liver to excrete some of the lipids. Group C had the highest increase in weight on comparison with the control. There was no significant difference between those of group F and group E (control). Also, there was no significant difference between the weights of other organs checked on comparing them with those of the control as shown in **Table 3**.

Histopathology

No death or clinical signs of toxicity such as locomotor activity alteration and piloterector were observed in any of the groups during the experimental procedures. Histopathological changes were checked in the heart, liver, kidney, intestine and spleen of all the rats fed. There was no visual lesion observed in the tissues of group E, A, D and F animals at a concentration of 5% of the oil. There is severe

Table 3 Weight of rat organs after 12 weeks of feeding experiment.

Organ	A (g)	B (g)	C (g)	D (g)	E (g)	F (g)
Liver	12.12 ± 0.10 a	12.62 ± 0.43b	13.22 ± 0.10b	12.81 ± 0.24b	11.01 ± 0.12a	11.11 ± 1.20a
Intestine	2.12 ± 1.30 a	1.89 ± 2.20a	2.01 ± 0.51a	1.61 ± 0.11a	1.91 ± 0.10a	1.75 ± 0.10a
Spleen	0.91 ± 1.10 a	1.01 ± 0.07a	1.11 ± 0.30a	0.89 ± 0.51a	1.00 ± 0.01a	1.10 ± 0.70a
Heart	1.20 ± 1.40 a	1.01 ± 0.21a	0.95 ± 3.10a	0.99 ± 0.52a	1.10 ± 2.10a	1.21 ± 0.10a
Kidney	1.81 ± 0.11 a	1.21 ± 0.33a	2.10 ± 0.04a	1.75 ± 2.00a	1.98 ± 0.01a	1.80 ± 1.00a

Means ± standard deviation of five determinations. Data in a row with different letters are statistically different ($P \leq 0.05$) according to DMRT.

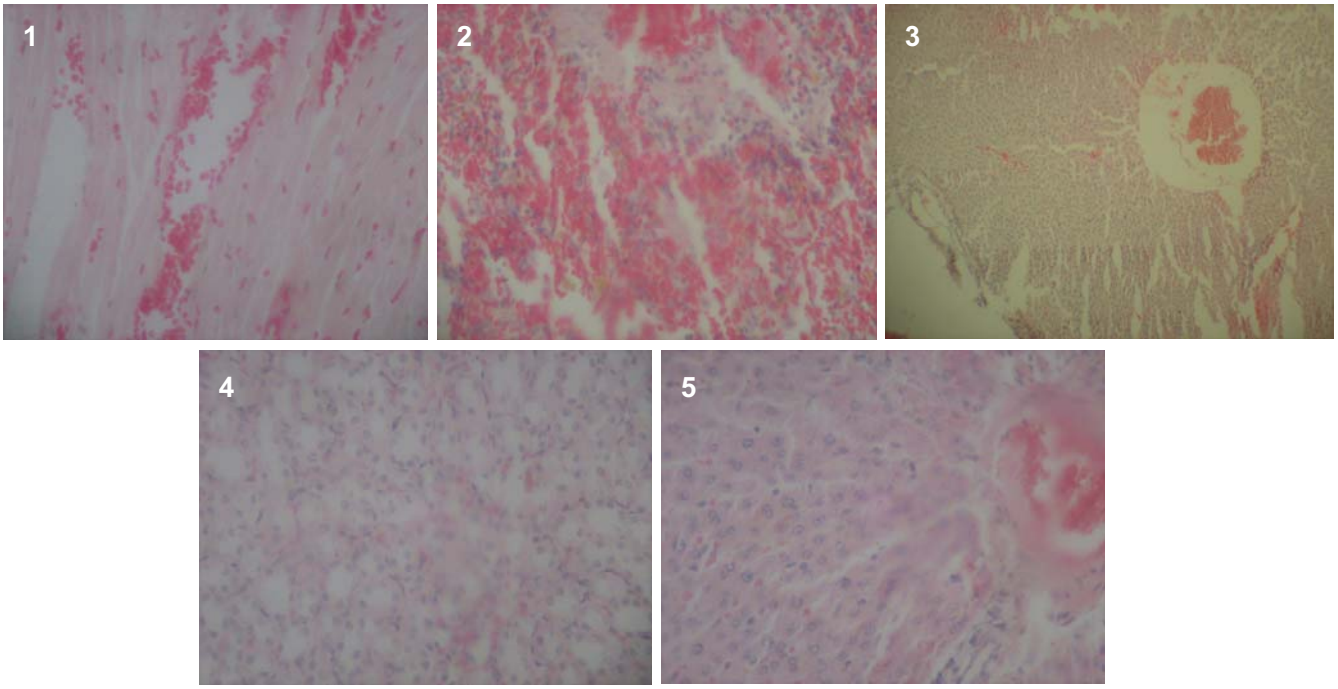

Fig. 1 Photomicrograph of the heart of group C rat showing severe myofibre haemorrhage. **Fig. 2** Photomicrograph of the spleen of group C rat showing very severe congestion. **Fig. 3** Photomicrograph of the heart of group C rat showing diffuse congestion of the parenchyma. **Fig. 4** Photomicrograph of the spleen of group B rat showing very mild congestion. **Fig. 5** Photomicrograph of the spleen of group B rat showing marked central venous congestion.

Table 4 Haematological parameters of rats after 12 weeks of feeding experiment.

Parameter	A	B	C	D	E	F
PCV (%)	40.10 ± 0.5a	29.21 ± 0.5b	39.00 ± 0.1a	37.00 ± 0.1a	32.00 ± 0.7c	36.00 ± 0.9c
Rbc x 10^6/l	7.14 ±0.6a	5.08 ± 0.1b	6.78 ± 0.1b	6.56 ± 0.2b	5.41 ± 0.3b	5.74 ± 0.1b
Wbc x 10^3/mm^3	6.60 ± 0.5a	5.90 ± 0.1a	3.40 ± 2.0b	7.10 ± 0.5a	2.90 ± 0.2b	6.00 ± 0.1a
Platelet (10^9/l)	138000 ± 5a	123000 ± 2a	107000 ± 2a	120000 ± 7a	75000 ± 4b	13800 ± 1b
MCV (fl)	56.21 ± 0.1a	57.01 ± 0.4a	57.00 ±0.4a	56.11 ± 0.1a	59.00 ± 0.1a	57.00 ± 0.1a
MCHC (g/dl)	33.00±0.5a	32.00 ±0.1a	32.00 ±0.1a	33.00 ±0.1a	32.00 ± 1.0a	32.00 ± 0.8a
Plasma Protein (g/dl)	7.30 ± 0.7a	6.50 ± 0.4a	7.20 ± 0.1a	6. 50± 0.1a	6.90 ± 0.5a	6.70 ± 0.1a
Fibrogen (mg/dl)	0.30 ±1.0a	0.30 ± 0.1a	0.30 ± 0.1a	0.30 ± 0.1a	0.40 ± 0.1a	0.20 ± 0.4a
Lymphocytes (%)	84.00 ± 1.0a	85.00 ± 7.0a	87.00 ± 1.0a	88.00± 1.0a	78.00 ± 5.0a	90.00 ± 5.0a
Segmented neutrophiles(%)	13.00 ± 0.3a	11.00 ± 1.0a	11.00 ± 0.2a	8.00± 0.1a	20.00 ± 0.1b	6.00 ± 1.0a
Band neutrophiles(%)	1.00 ± 0.1a	1.00 ± 0.1a	1.00 ± 0.3a	1.00 ± 0.0a	1.00 ± 0.2a	1.00 ± 0.1a
Monocytes (%)	2.00 ± 0.5a	2.00 ± 0.1a	2.00 ± 0.5a	2.00 ± 0.1a	2.00 ± 0.1a	2.00 ± 0.5a
Eosinophils (%)	1.00 ± 0.0a	1.00 ± 0.0a	1.00± 0.0a	1.00 ± 0.0a	1.00 ± 0.0a	1.00 ± 0.0a
Hb (g/dl)	13.20 ± 3.0a	9.40± 0.3b	12.70 ± 0.1a	12.40 ± 0.1a	10.30 ± 0.1b	10.60 ± 0.3b

Means ± standard deviation of five determinations. Data in a row with different letters are statistically different ($P \leq 0.05$) according to DMRT.

myofibre haemorrhage in the heart and very severe congestion in the spleen of the animals in group C as shown in **Figs. 1** and **2**. Diffused congestion of the parenchyma was also noticed in the kidney of the group C animals (**Fig. 3**). Very mild congestion was also found in the kidney of group B animals (**Fig. 4**) with the liver showing a marked central venous congestion (**Fig. 5**).

Haematology

The result of the haematology assay is presented in **Table 4**. There are significant differences in PCV, RBC, Hb and segmented neutrophiles in some of the test groups. The PCV value (40.10 ± 0.50%) of group A was higher than that of the control (37 ± 0.10%) with group B being the lowest (29.21 ± 0.50%). The RBC of group A and group C were found to be a little higher than the value obtained for the control while others were below the value of the control (group E). No changes were observed in eosinophiles, monocytes or band neutrophiles. Also, there were no distinct differences in the values obtained for MCHC, fibrogen and plasma protein. The decrease in RBC, Hb and PCV values without a significant effect on MCV and MCHC indicated normocytic normochromic anaemia in rats fed with 5% HC (group D). In comparison with the control the WBC of group fed with HC was also found to be lower than the value of the control. It was the least for all tested oils and this was also reflected in the value of its lymphocytes, which was also the lowest.

Biochemical parameters

The biochemical assay revealed significant increase in the activity of GGT in groups A, B and C. There was also an increase in the concentration of urea and cholesterol in all the test groups when compared with the control. There was a decrease in the activity of ALT in group A and D. The amount of ALP was also found to decrease in group B and F while group A recorded the highest concentration of protein and globulin.

A rise in the activity of GGT, urea and cholesterol concentration suggests damage to the liver but the levels of protein and albumin recorded did not support this. Even the level of ALT, AST and ALP were found to be low and with no significant difference in the groups. ALT, AST and ALP are also biomarkers of damage in organs, which showed no level of damage in this study.

Blood lipid values are risk markers for obesity, diabetes and coronary heart diseases (MacQueen *et al*. 2007). From our study we noted an increase in the level of total choles-

Table 5 Biochemical parameters of rats after 12 weeks of feeding experiment.

Parameter	A	B	C	D	E	F
Albumin (mg/l)	3.80 ± 0.20a	3.60 ± 0.10a	3.20 ± 0.50a	3.40 ± 0.10a	3.60 ± 0.01a	3.80 ± 0.04a
Creatinine (mmol/l)	0.80 ± 0.50a	0.70 ± 0.10a	0.60 ± 0.30a	0.60 ± 0.50a	0.60 ± 0.05a	0.70 ± 0.30a
TP (mg/l)	8.70 ± 0.30a	6.70 ± 0.10a	6.00 ± 0.30a	6.20 ± 0.10a	6.10 ± 0.05a	6.00 ± 0.10a
Globulin	4.50 ± 1.00a	3.10 ± 0.50a	2.80 ± 0.50a	2.80 ± 0.10a	2.50 ± 0.10a	2.20 ± 0.20a
AST (ui/l)	211.00 ± 9.00a	202.00 ± 21.00a	211.00 ± 11.00a	201.00 ± 8.00a	229.00 ± 4.00a	217.00 ± 9.00a
ALT (ui/l)	91.00 ± 11.00a	116.00 ± 8.00a	117.00 ± 14.00a	85.00 ± 31.00a	115.00 ± 3.00a	114.00 ± 8.00a
GGT (ui/l)	10.00 ± 0.30a	7.00 ± 0.20b	4.00 ± 0.10c	2.00 ± 0.20c	1.00 ± 0.01c	3.00 ± 0.01c
Total bilirubin	0.30 ± 0.20a	0.30 ± 0.10a	0.10 ± 0.02a	0.20 ± 0.05a	0.30 ± 0.01a	0.20 ± 0.10a
TG (mg/l)	35.00 ± 4.00a	30.00 ± 5.00a	38.00 ± 1.00a	48.00 ± 3.00a	36.00 ± 1.00a	40.00 ± 8.00a
Urea (mmol/l)	85.00 ± 5.00a	46.00 ± 3.00b	73.00 ± 5.00a	40.00 ± 3.00b	25.00 ± 1.00c	38.00 ± 5.00b
TC (mg/l)	164.00 ± 25.00a	100.00 ± 31.00a	149.00 ± 23.00a	87.00 ± 10.00b	55.00 ± 1.00c	68.00 ± 5.00c
HDL (mg/l)	45.00 ± 7.00a	55.00 ± 6.00a	40.00 ± 11.00a	42.00 ± 6.00a	30.00 ± 5.00a	38.00 ± 5.00a
LDL (mg/l)	110.00 ± 2.00a	41.00 ± 8.00b	98.00 ± 6.00a	38.00 ± 5.00b	18.00 ± 9.00c	20.00 ± 11.00b
Glucose (mg/l)	129.00 ± 4.00a	138.00 ± 11.00a	124.00 ± 10.00a	140.00 ± 8.00a	170.00 ± 5.00a	157.00 ± 5.00a
ALP (ui/l)	14.00 ± 5.00a	10.00 ± 3.00a	16.00 ± 1.00a	15.00 ± 5.00a	14.00 ± 1.00a	8.00 ± 1.00a

Means ± standard deviation of five determinations. Data in a row with different letters are statistically different ($P \leq 0.05$) according to DMRT.
TP = Total Protein; AST = Aspartate Transaminase; ALT = Alanine Transaminase; GGT = Gamma Glutamyl Transferase; TG = Triglyceride; TC = Total Cholesterol; HDL = High Density Lipoprotein; LDL = Low Density Lipoprotein; ALP = Alkaline Phosphate.

terol, LDL and HDL in all the test groups. The diet had altered lipid concentrations causing marked hyperlipidemia, as shown in **Table 5**. This resulted in a high atherogenic index of the control and the test groups. Only the triglyceride level was found to be similar in all groups except for group D fed with HC oil. Hyperlipidemia, including hypercholesterolemia and hypertriglyceridemia is a major risk factor for the development of cardiovascular diseases while the reduction of circulatory triglycerides, total and LDL cholesterol is a primary step in the prevention of vascular diseases (Corrina *et al*. 2006).

The type of compounds present in these oils influences the profile of hepatic lipid accumulation and this influence depends on the animal species studied (Ros 2000). The increase in concentration of cholesterol may be due to the slow uptake of chylomicron remnants originating from saturated fatty acid in the oil. The insignificant difference in the triglyceride level is likely to be due to hepatic lipid synthesis in these test groups surpassing the lipoprotein lipase triglyceride hydrolysis, so that the accumulation in the liver becomes more evident than its effect in the blood. Chronically, this diet can induce cellular lipotoxicity.

In summary, the studied oils of HC and *B. unijugata* kernels show no lesions in the organs of the rats whereas the oil from the aril of *B. unijugata* and the combination of arils and kernels have a deleterious effect on some of the studied organs of the rats. There is also the possibility of cellular lipotoxicity based on the alteration of lipid profile obtained. This has obvious implications for dietary choice and long term health.

ACKNOWLEDGEMENTS

The authors would like to thank the Department of Chemistry, Botany, Veterinary Medicine and University College Teaching Hospital (UCH), University of Ibadan for their facilities.

REFERENCES

Ajayi IA, Dawodu FA, Adebowale KO, Oderinde RA (2004) A study of the oil content of Nigerian grown *Monodora myristica* seeds for its nutritional and industrial application. *Pakistan Journal of Industrial Research* **47 (1)**, 60-65

Ajayi IA, Oderinde RA, Ogunkoya A, Egunyomi A, Taiwo VO (2006) Chemical analysis and preliminary toxicological evaluation of *Garcinia mangostana* seeds and oil. *Food Chemistry* **77**, 431-437

Botham KM, Zheng X, Napolitano M, Avella M, Cavallari C, Rivabene R, Bravo E (2003) The effects of dietary n-3 polyunsaturated fatty acids delivered in chylomicron remnants on the transcription of genes regulating synthesis and secretion of very-low-density lipoprotein by the liver: modulation by cellular oxidative state. *Expert in Biological Medicine (Maywood)* **228**, 143-151

Burkill HM (1994) *The Useful Plants of West Tropical Africa* (2nd Edn, Vol 2), Royal Botanic Garden, Kew, pp 84-85

Burkill HM (2000) *The Useful Plants of West Tropical Africa* (2nd Edn, Vol 2), Royal Botanic Garden, Kew, pp 11-13

Corrina B, Anjali S, Frank H, Gabriele I, Klaus E (2006) Effect of proteins from beef and turkey meat on plasma and liver lipids of rats compared with casein and soy protein. *Nutrition* **22**, 1162-1170

Duncan DB (1955) Duncan multiple range and multiple F tests. *Biometrics* **11**, 1-42

Lee S-H, Park H-J, Chun H-K, Cho S-Y, Jung H-J, Cho S-M, Kim D-Y, Kang M-S, Lillehoj H-S (2007) Dietary phytic acid improves serum and hepatic lipid levels in aged ICR mice fed a high-cholesterol diet. *Nutrition Research* **27**, 505-510

MacQueen HA, Sadler DA, Moore SA, Daya S, Brown JY, Shuker DEG, Seaman M, Wassif WS (2007) Deleterious effect of a cafeteria diet on the liver of non-obese rats. *Nutrition Research* **27**, 38-47

Odebiyi OO, Sofowora EA (1978) Phytochemical screening of Nigerian medicinal plants. *Lloydia* **41**, 234-235

Ros E (2000) Intestinal absorption of triglyceride and cholesterol. Dietary and pharmacological inhibition to reduce cardiovascular risk. *Atherosclerosis* **151**, 357-379

Schalm OW, Jain NC, Carroll GH (1975) *Veterinary Haematology* (3rd Edn), Wacsworth Publishing Co. Inc, Belmont, pp 5-8

Skim F, Lazrek H, Amri H, Kaaya A, Jana M (1998) Toxicological studies on *Globularia alypum* and *Zygophyllum gaetulum* in rats. *Phytotherapy Research* **12**, 592-594

Yaqoob P, Sherrington EJ, Jeffery NM, Sanderson P, Harvey DJ, Newsholme EA, Calder PC (1995) Comparison of the effect of a range of dietary lipids upon serum and tissue lipid composition in rat. *International Journal of Biochemistry and Cell Biology* **27**, 297-310

Yang R-L, Le G-W, Li A-L, Zheng J-L, Shi Y-H (2006) Effect of antioxidant capacity on blood lipid metabolism and lipoprotein lipase activity of rats fed with a high-fat diet. *Nutrition* **22**, 1185-1191