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Short-term toxicological evaluation of *Neocarya macrophylla* seed oil based diets in albino rats

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Abstract

The nutritional and toxicological evaluation of *Neocarya macrophylla* seed oil (NMSO) was carried out in order to determine the suitability of the oil for nutritional purposes using wistar rats. A total of 28 wistar albino rats were divided into four groups of 7 rats each. Group A was fed with an industrially purchased feed and group B with a control diet compounded with 10 % (w/v) groundnut oil containing 22.84±0.12 % of protein, 4.80±0.01 % of fat, 44.8±0.14 % of carbohydrate and 1328.11±0.69 of energy value. Groups C and D were fed with diet compounded with 5 % (w/v) and 10% (w/v) NMSO respectively. After 6 weeks, the rats were sacrificed and their blood samples and organs collected for analysis. The growth parameters of the rats in the four groups appeared considerably good at the end of the experiment. Rats in group C had the highest weight gain. No significant difference was observed between the average values obtained for LDL in the total lipid profile analysis for the four groups. The blood biochemistry result showed no significant difference in the average values of total protein (7.90±0.66-8.07±0.32), albumin (2.63±0.85-3.10±0.72), globulin (4.80±0.10-5180±0.89), AST (40.00±1.73-41.67±13.22), ALT (28.00±2.65-30.00±3.00) and creatinine (0.73±0.23-0.77±0.21). No significant difference was also observed in the haematological parameters of the rats across the treatments. No major lesion was found in the kidney and heart of both the control and experimental rats. NMSO at 10 % (w/v) appeared to be non-toxic and might cheaply replace conventional vegetable oils such as groundnut oil.

Key words: *Neocarya macrophylla*, seed oil, haematology, toxicological evaluation

Introduction

Neocarya macrophylla (formerly *Parinari macrophylla* Sabine) commonly known as "*Gawasa*" in Hausa language belongs to *Chrysobalanaceae* family. It is grown in arid and semiarid regions mainly in the Western part of Africa. It is a shrub or small tree that is 6-10 m high with densely pubescent and russet brown stems and alternate or ovate leaves 10-25cm long (1). The plant is semi-cultivated in Northern part of Nigeria and its fruits are harvested from the ground (2). The fruits are used in variety of ways. Many are eaten fresh or boiled with cereals. Fragrant syrups are prepared and proved to be much stronger than some fruit juice (2). It is one of the seeds that are rich in protein, but lesser-known, therefore underexploited (3). *N. macrophylla* kernel oils can be classified within the tree crop source and are native to Western Africa and Central America including Ghana, Guinea, Guinea Bissau, Ivory Coast, Liberia, Mali, Niger, Nigeria, Senegal, Sierra Leone, Gambia and Panama (2). The ratio of the endocarp to the kernel is about 85:15 %. *N. macrophylla* kernel is edible and has been recorded as containing 62 % oil, while 9 % has been found in the endocarp (4). The seeds provide energy, dietary fibre, protein, minerals and fatty acids required for human health (5). Pharmacologically, the decoction of the bark and leaves are used as mouth wash, internal troubles and for inflamed eye. The leaves may also be chewed or applied topically for the same end. The fruits are used in a variety of ways. Many are eaten fresh or are boiled with cereal. *N. macrophylla* nuts are usually roasted and enjoyed like cashews or almonds. Some are consumed as snacks, others

mixed into cooked dishes, and a few are pressed to yield cooking oil and the leaves are used medicinally for toothache and mouthwash (6). Previous studies have reported that the seeds are of high food value with about 40-60 % oil and 21-25 % protein contents (7). The defatted seed meal contains 61 % protein. The seeds are good source of certain amino acids such as lysine, valine and phenylalanine and others which are important for balancing the deficiency of these essential amino acids in cereal-based diets (7).

N. macropylla is a plant employed in traditional medicine to manage pain conditions in Northern Nigeria (8). It is also used in treating diarrhea, asthma, dysentery, skin infections, cancer, pulmonary troubles, ear and eye infections, tooth decay, snakebite, pain, inflammation and skin infections (9). Some preliminary phytochemical screening and physico-chemical studies of the seed oil (9) as well as the antimicrobial studies on the fruit and root bark of the plant (10) have been previously undertaken. The leaves have anthelminthic activities (11). The nutritional and anti-nutritional profiles of the seeds have also been reported (12)..The utilization of *N. macrophylla* seed oil in diets and other food supplements has not been investigated. Even though this work has been presented in a conference in previous year, it has not been published in any academic journal. The main objective of the study was to assess the toxicity effect, nutritional potential and growth performance of *N. macrophylla* seed oil (NMSO) on rat. This is part of our efforts to bring into focus the many lesser known seed oils for nutritional and industrial purposes (13, 14, 15, 16).

Materials and Methods

Preparation of *N. macrophylla* seed oil

N. macrophylla seeds were obtained from Junju town, Niger Republic and were identified and authenticated at the Biological Science Department, Bayero University, Kano. The seeds were selected, cleaned andkept at room temperature. They were well dried and milled using a laboratory scale hammer miller prior to extraction. The kernels were pulverized to fine powder to increase the extent of extraction. Oil was extracted from the seed flour by soxhlet extraction method using n-hexane (b.pt 67-68 °C) as the solvent continuously for 8 h. After extraction, the solvent was distilled off completely from the oil. The oil obtained was stored in a properly labelled glass container for further study.

Feed formulation and preparation

The diets used in this study were formulated to meet the entire nutrient requirement for young rats. They were prepared according to the formula and procedure used by Toyomizu *et al.* (17) and Ajayi *et al.* (15) with little modification and labelled A, B, C and D according to the experimental groups of rats. Diet A was a standard feed bought from Ladokun Feed Lmited, Ibadan, Nigeria and served as control I. The basic ingredients used to compound other diets were: maize (40 %), soybeans (18.00 %), bone (3.2 %), salt (0.80 %), groundnut cake (4.5 %), palm kernel cake (7.08 %), wheat (7.08 %), corn bran (7.08 %), groundnut oil (10 % w/v), and oyster shell (2.26 %). 5 % and 10 % of NMSO were used to replace the groundnut oil (10 % w/v) in the preparation of diets C and D which served as the experimental diets.

Experimental animals, diets and feeding

Twenty eight albino rats (weighing between 55 and 100 g) were purchased from the experimental animal house of Veterinary Department, University of Ibadan, Nigeria.

The animals were allowed to acclimatize for one week and maintained on the standard normal diet (Ladokun Feeds Limited, Ibadan, Nigeria) with water *ad-libitum* in the animal house under normal room temperature before the commencement of the experiment. The animals were then distributed randomly into four different groups A, B, C, D of seven animals each. Group A and B rats (control I and II) were fed with the standard feed (Ladokun Feed Limited, Ibadan, Nigeria) and diet compounded with 10 % ground nut oil respectively while groups C and D (experimental groups) received diet compounded with 5 % and 10 % NMSO respectively. The animals were allowed to acclimatize for one week and maintained on the standard normal diet (Ladokun Feeds Limited, Ibadan, Nigeria) with water *ad-libitum* in the animal house under normal room temperature

before the commencement of the experiment. The animals were fed for a period of 6 weeks with the compounded diets and they had unrestricted access to water before sacrifice. The physical appearance of the rats was monitored, the feed intakes were recorded daily and the body weight of each rat was recorded weekly for the period of the experiment. Animals were sacrificed after 14-16 h overnight fast on the last day of the experiment. Blood samples for haematology, biochemical parameters and tissue samples for histopathology were taken at the end of the six weeks.

Nutritional assessment (growth performance, feed utilization and survival)

The growth performance parameters were measured according to the methods described by Oleva- Novoa *et al.* (18) with a little modification by Ajayi *et al.* (16). Mean weight gain (MWG) was calculated as the difference between the initial and final weight divided by the number of the surviving rat at the end of the feeding period. Specific growth rate (SGR) is the relationship of the difference in the weight of the rat within the experimental period. Feed conversion ratio (FCR) was determined by dividing the total weight of the food given by the total weight gained by the rat over a period of time while feed intake (FI) was calculated as the addition of daily mean feed intake of the rat during the period. Average daily growth (ADG) was calculated as the difference between the final weight and the initial weight divided by the number of days for the experiment. Feed efficiency ratio= weight gain/ Total feed intake. Relative weight of organs = (Weight of organ / Rat's body weight) X 100 (19).

Haematological examination

3 ml of blood were collected by cardiac puncture into heparinized vials and stored at 10 ^{o}C for analysis the same day haematological examination. The packed cell volume (PCV), haemaglobin (Hb) concentration, red blood cell (RBC) and white blood cell (WBC) counts were determined using standard techniques as described by Dacie and Lewis (20). The differential WBC counts mean corpuscular volume (MCV) and mean corpuscular haemaglobin concentration (MCHC) were calculated (20, 21).

Blood biochemical analyses

Blood sample was kept at room temperature for 30 min to clot. Afterwards, the test tube containing the clotted blood sample was centrifuged at 3000 r/min for 10 min using a tablecentrifuge to enable a complete separation of the serum from the clotted blood. The clear serum supernatant was then carefully aspirated with syringe and needle and stored in aclean sample bottle for the biochemical tests. The values of blood glucose, total serum protein, serum albumin, serum cholesterol, serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST), serum alkaline phosphatise (ALP), serum urea and serum creatinine were determined following standard laboratory procedures (22).

Plasma lipid profiles

Plasma concentrations of total cholesterol and triglycerides were determined with commercial kits (Spin React S.A., Santa Colona, SantEsteve De Bas, Spain). High density lipoprotein (HDL) cholesterol and triglycerides were determined in plasma with the same commercial kits for total cholesterol andtriglycerides after very low density lipoproteins (VLDL) and low density lipoprotein (LDL) were precipitated with heparin–MnCl2 solution, . LDL cholesterol was calculated through VLDL cholesterol using the method of Afolabi *et al.* (23).

Histological studies

Histological analyses of the heart, liver and kidney samples were carried out.Small portions of these tissues already harvested, weighed and stored in 10 % formalin were fixed and put through series of dehydration in graded concentration of xylene. They were embedded in wax, sectioned at 5 μand transferred to clean glass slides. The thin sections were stained with haemotoxylin and eosin (H and E) dyes for examination under the light microscope for histological changes following the method outlined by Jain (21).

Statistical analysis

The data obtained during the feeding trial were expressed as mean values±standard deviation. Organ weights, biochemical and haematological determinations and others were subjected to the statistical analysis of variance (ANOVA) using SPSS (Statistical Package for Social Science) while the difference among individual means was determined by the use of Duncan Multiple Range Test. A probability level less than 5 % ($p \leq 0.05$) was considered significant.

Results and Discussion

Physical appearance of rats

No offensive odour was observed, and the eyes, mouths and hairs of the animals appeared normal in the four groups, although the animals in group D (10 % (w/v) *N. macrophylla* seed oil) were observed to excrete more than the animals in the other groups.

Effect of *N. macrophylla* seed oil based formulated feed on growth performance, feed intake and survival rate of rats

The mean body weight, average weight gain, survival rate and feed intake of the rats treated with NMSO are presented in Table 2. There was no significant difference in body weight increase between controls and experimental groups with groups A & B having the highest weight gain. The feed intake is relatively higher and comparable among the groups. The feed conversion ratio, specific growth rate and daily growth rate are also comparable. Group C had a lower survival rate of 85.71 % compared to 100 % in other groups due to the loss of one animal. The effect of feed consumption on body weight gain has already been studied (24). The results of this study are in line with the report in literature. This study showed positive changes in the body weight of the animals; it seemed that the consumption of the feed compounded with 10 % NMSO had no adverse effects on the body weights and appetite of the animals. Weekly physical appearance of the rats throughout the study period was normal. No offensive odour was observed, and the eyes, mouths and hairs of the animals appeared normal in the four groups.

Table 1: Physical appearance of the rats per week

Weeks	Eye				Mouth				Hair			
	A	B	C	D	A	B	C	D	A	B	C	D
1	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
2	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
3	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
4	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
5	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
6	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++

+++: Normal. Group A: Standard feed; Group B: 10 % (w/v) groundnut oil; Group C: 5 % (w/v) *N. macrophylla* seed oil; Group D: 10 % (w/v) *N. macrophylla* seed oil

Table 2: Average feed consumed by rats weekly (g)

No. of weeks	Group A	Group B	Group C	Group D
1	106.86±11.34^{b}	134.00±16.30^{b}	95.29±15.85^{e}	116.29±26.93^{b}
2	106.29±9.64^{b}	118.86±4.34^{cd}	108.14±8.76^{d}	114.57±4.50^{b}
3	105.57±7.64^{b}	113.71±5.35^{d}	114.29±5.74^{cd}	115.57±5.22^{b}
4	107.71±3.95^{b}	118.43±0.98^{cd}	119.86±0.38^{bc}	119.71±0.76^{b}
5	113.57±14.14^{b}	125.57±5.65^{bc}	127.14±4.88^{b}	125.43±4.69^{b}
6	138.29±8.96^{a}	150.71±5.77^{a}	153.00±9.97^{a}	147.14±11.31^{a}
Feed conversion ratio (FCR)	6.43	7.46	5.92	6.98
Average weight increase of rats (g)				
0	68.86±17.12^{a}	76.57±3.78^{a}	77.71±7.63^{a}	78.29±9.16^{a}
1	84.71±21.46^{a}	92.00±4.69^{a}	97.86±6.36^{a}	98.29±9.95^{a}
2	103.43±29.73^{a}	113.29±5.62^{a}	125.14±15.39^{a}	124.29±13.41^{a}
3	122.00±31.12^{a}	128.00±11.53^{a}	137.86±10.72^{a}	136.86±10.72^{a}
4	126.71±24.98^{a}	135.43±14.09^{a}	146.29±34.97^{a}	151.86±18.39^{a}
5	158.43±32.72^{a}	161.00±20.42^{a}	185.33±12.01^{a}	169.86±17.18^{a}
6	174.29±34.68^{a}	178.57±22.63a	199.00±15.75^{a}	184.14±12.48^{a}
Mean Specific growth rate (SGR)	2.21	2.02	2.24	2.04
Mean Daily growth rate	2.51	2.43	2.88	2.52
Survival (%)	100.00	100.00	85.71	100.00

Values are expressed as mean±SD. Values on the same row having the same letter as superscripts are not significantly different (*P < 0.05*). Group A: Standard feed; Group B: 10 % (w/v) groundnut oil; Group C: 5 % (w/v) *N. macrophylla* seed oil; Group D: 10 % (w/v) *N. macrophylla* seed oil

Table 3: Mean weight of organs (g)

Tissue	Group A	Group B	Group C	Group D
Kidney	1.11±0.17^{b}	1.21±0.07^{ab}	1.28±0.10^{a}	1.29±0.04^{a}
Brain	1.56±0.10^{a}	1.50±0.16^{a}	1.60±0.11^{a}	1.54±0.17^{a}
Liver	6.81±0.94^{a}	6.79±0.81^{a}	7.73±0.69^{a}	6.87±0.77^{a}
Heart	0.60±0.16^{ab}	0.57±0.05^{ab}	0.65±0.05^{a}	0.50±0.08^{b}
Lungs	1.50±0.51^{a}	1.36±0.21^{a}	1.30±0.17^{a}	1.19±0.18^{a}
Spleen	0.74±0.17^{a}	0.91±0.23^{a}	0.90±0.30^{a}	0.80±0.11^{a}

Values are expressed as mean±SD. Values on the same row having the same letter as superscripts are not significantly different (*P < 0.05*). Group A: Standard feed; Group B: 10 % (w/v) groundnut oil; Group C: 5 % (w/v) NMSO; Group D: 10 % (w/v) NMSO

Weight of organs

The mean weight of the organs of the different groups of rat collected for pathological analysis is presented in Table 3. There was no significant difference in organ weight increase between control and experimental groups. An average

liver weight of 6.81±0.94 g; 6.79±0.81 g; 7.73±0.69 g and 6.87±0.77 g was obtained respectively for the control and experimental groups. These values are higher than 4.47±0.51 g and 5.63±0.98 g reported for rats fed with *Cucumeropsis mannii* seed oil (25). Organ weight is an important factor of physiological and pathological status in animals. The relative organ weight is fundamental to establish whether the organ was exposed to any injury or not. The heart, liver, kidney, spleen, and lungs are the primary organs affected by metabolic reaction caused by toxicant (26). The liver, being a key organ in the metabolism and detoxification of xenobiotics, is vulnerable to damage induced by a huge variety of chemicals. The absence of significant difference in the parameter obtained in this study is an indication that the weights of the organs are not affected by 10 % NMSO.

Haematological analysis

The assessment of haematological parameters could be used to reveal the deleterious effect of foreign compounds on the blood constituents of animals. They can also be used to determine possible alterations in the levels of bio-molecules, metabolic products; haematology, normal functioning and histomorphology of the Organs (27). Presented in Table 4 are thehaematological parameters of the control and experimental animals. There were significant differences ($P \leq 0.05$) in the platelet and an absolute eosinophyl value. Platelet had a highest value of $28.35 \pm 2.98 \times 10^4$; $22.10 \pm 6.00 \times 10^4$ in control groups compared to $16.40 \pm 9.98 \times 10^4$ and $17.05 \pm 4.14 \times 10^4$ obtained respectively in the experimental groups. Absolute eosinophyl value of 178.50±27.58 was highest in the10 % NMSO. Mean MCV, MCH and MCHC which are RBC indices that are used in classifying types of anaemia did not show any major significant changes in the experimental animals when compared with the controls. All other haematological parameters obtained in the experimental groups did not show any significant difference ($P \leq 0.05$) when compared with those in the control groups.

Blood biochemistry analysis

The result of the blood biochemistry analysis is shown on table 4. No significant difference in the average values of total protein, albumin, globulin, albumin/globulin ratio, AST, ALT, and creatinine was observed in all four groups under study. Within the hepatocytes, high levels of AST and ALT are usually present and as the hepatocytes membrane integrity is disturbed during hepatocellular cell injury, plasma levels rise (28). ALT is a cytotoxic enzyme found in very high concentration in the liver (29), and an increase of this specific enzyme indicates hepatocellular damage. Increase in ALT and AST are also clinical indication of diagnosing state of damage done to visceral organ by toxic substance or infection. Serum urea and creatinine are indicators of kidney injury and are elevated in renal toxicity (30). The experimental groups compared favourably with the control groups as the values obtained for all the parameters are not significantly different ($P \leq 0.05$). The absence of significant changes in ALT and other related indicators such as AST, total protein, albumin and A/G ratio may suggest that the hepatotoxic effect of the oil on the rats was mild. The above result suggests that the consumption of 10 % NMSO in moderate quantities might not be dangerous.

Total lipid profile analysis

Presented on Figure 1 is the total cholesterol and total triacyglycerol of the hearts of the control and experimental rats. The average values of total cholesterol and LDL are not significantly different in all four groups. The average values of total cholesterol (67.33±3.06) in 10 % NMSO oil is higher than 56.67±16.01 obtained for 5 % NMSO but comparable with 67.00±7.21in the control with ground nut oil. HDL value of 34.67±1.52 was highest in 10 % NMSO group than other groups. Triglyceride (47.00±7.55) is lower when compared to 54.67±9.87 for the groundnut oil group but compared greatly with the 5 % NMSO group. LDL showed no significant difference ($P \leq 0.05$) in both experimental and control groups. Alterations in levels of serum lipids in animals offer information on the effect of diet on lipid metabolism as well as predisposition to coronary heart diseases (30). HDL is essential in the transportation of cholesterol from tissues to the liver where it is metabolized (31) and it is also considered as good cholesterol with

antiatherogenic properties. HDL constitutes a protective factor against cardiovascular diseases. Thus, the increase in HDL and decrease in triglyceride levels of the rats fed with 10 % NMSO diet for 6 weeks could reduce susceptibility to cardiovascular disease. Similar results were reported by Nwozo *et al.* (32) for defatted *Detarium senegalense* seed-based diet.

Table 4: Result of haematological analysis

Parameters	Group A	Group B	Group C	Group D
PCV (%)	40.25±3.59[a]	39.75±6.55[a]	45.25±5.25[a]	42.25±3.59[a]
Hb (g/dL)	13.35±0.97[a]	13.08±2.40[a]	14.85±1.75[a]	13.83±0.89[a]
RBC (10^6/μL)	6.66±0.41[a]	6.55±1.43[a]	7.58±0.94[a]	7.00±0.46[a]
WBC (10^3/μL)	4637.50±534.44[a]	5512.50±759.80[a]	4625.00±988.69[a]	5375.00±1002.08[a]
Platelet (x 10^4)	28.35±2.98[a]	22.10±6.0[ab]	16.40±9.98[b]	17.05±4.14[b]
Lymphocyte (%)	65.50±5.32[a]	68.00±3.74[a]	70.25±2.99[a]	65.75±4.35[a]
Neutrophyl (%)	31.00±6.27[a]	28.00±3.16[a]	26.25±3.10[a]	30.25±4.65[a]
Monocyte (%)	1.75±0.50[a]	2.00±0.82[a]	1.75±0.50[a]	2.50±1.00[a]
Eosinophyl (%)	1.75±0.96[a]	2.00±0.82[a]	1.75±0.50[a]	3.00±0.00[a]
MCV (fL)	60.37±1.79[a]	61.40±4.34[a]	59.78±0.98[a]	60.30±1.28[a]
MCHC (%)	33.21±0.78[a]	32.82±0.78[a]	32.81±0.25[a]	32.77±0.80[a]
MCH (pg)	20.03±0.25[a]	20.13±1.09[a]	19.60±0.18[a]	19.75±0.13[a]
Lymphocyte	3039.00±426.31[a]	3765.00±662.75[a]	3256.38±748.29[a]	3519.13±566.91[a]
Neutrophyl	1439.63±335.69[a]	1530.13±156.73[a]	1204.00±252.27[a]	1638.38±495.33[a]
Monocyte	79.25±17.15[a]	110.25±51.29[a]	84.63±35.48[a]	128.25±40.75[a]
Eosinophyl	79.63±40.06[b]	107.13±34.22[b]	80.00±27.68[b]	178.50±27.58[a]

Values are expressed as mean±SD. Values on the same row having the same letter as superscripts are not significantly different ($P < 0.05$). Group A: Standard feed; Group B: 10 % (w/v) groundnut oil; Group C: 5 % (w/v) NMSO; Group D: 10 % (w/v) NMSO

Table 5: Result of blood biochemistry analysis

Parameters	Group A	Group B	Group C	Group D
Total protein (g/dL)	7.90±0.66[a]	7.97±0.38[a]	7.97±0.29[a]	8.07±0.32[a]
Albumin (g/dL)	3.10±0.72[a]	2.93±0.76[a]	2.90±0.82[a]	2.63±0.85[a]
Globulin (g/dL)	4.80±0.10[a]	5.03±0.38[a]	5.07±0.55[a]	5.10±0.89[a]
Albumin/Globulin	0.60±0.17[a]	0.57±0.21[a]	0.53±0.25[a]	0.50±0.26[a]
AST (μL)	40.67±1.52[a]	40.33±2.52[a]	40.00±1.73[a]	41.67±3.22[a]
ALT (μL)	28.00±2.65[a]	29.00±3.46[a]	28.67±3.79[a]	30.00±3.00[a]
ALP (μL)	76.00±7.21[b]	92.00±19.47[ab]	103.00±3.00[a]	89.00±6.25[ab]
Urea (mg/dL)	15.53±1.17[ab]	16.67±0.57[a]	15.13±0.38[b]	16.93±0.64[a]
Creatinine (mg/dL)	0.73±0.23[a]	0.77±0.06[a]	0.77±0.06[a]	0.77±0.21[a]

Values are expressed as mean±SD. Values on the same row having the same letter as superscripts are not significantly different ($P < 0.05$). Group A: Standard feed; Group B: 10 % (w/v) groundnut oil; Group C: 5 % (w/v) NMSO; Group D: 10 % (w/v) NMSO.

Table 6: Summary of histopathology of tissues of the control and experimental rats

Organ	Heart	Kidney	Liver
Group A	There are a few foci of mild degeneration of cardiomyo-cytes evidenced by loss of striations. There is moderate congestion of coronary blood vessels.	There is mild congestion of interstitial renal blood vessels. The glomeruli, tubules and renal interstitium appear normal.	Hepatic plates are closely-packed. There are multiple foci of mild vacuolar change of hepatocytes.
Group B	There are a few foci of mild degeneration of cardiomyo-cytes evidenced by loss of striations. There is moderate congestion of coronary blood vessels.	There are locally extensive foci of mild sloughing off and flattening of epithelium of tubules in the renal medulla cortico-medullary junction.	There is moderate thinning of hepatic plates. There is widespread marked vacuolar change of hepatocytes (Hepatocytes have clear cytoplasmic vacuoles).
Group C	Cardiomyocytes appear normal. No visible lesion.	The glomeruli, tubules and renal interstitium appear normal.	There are multiple foci of moderate thinning of hepatic plates with consequent dilation of hepatic sinusoids. There are random foci of single-cell hepatocellular necrosis; this suggests certain level of toxicity in te presence of NMSO.
Group D	Cardiomyocytes appear normal. No visible lesion.	There are a few foci of flattening of tubular epithelium.	There are multiple foci of moderate thinning of hepatic plates with consequent dilation of hepatic sinusoids. There are random foci of single-cell hepatocellular necrosis.

Histological study

The histopathology of the rats after the experiment is summarized on Table 6 while the photomicrographs observed with microscopy (x400) are shown in figs. (2- 4). The hearts and the kidney of the experimental groups appear more normal than those of the control groups, as there is no degeneration of cardiomyocytes in the hearts of the animals in the experimental groups. There are random foci of single-cell hepatocellular necrosis in the kidney of both the experimental groups. Multiple foci of moderate thinning of hepatic plates with consequent dilation of hepatic sinusoids as well as random foci of single-cell hepatocellular necrosis were observed in the liver of the experimental groups while multiple foci of mild vacuolar change of hepatocytes and widespread marked vacuolar change of hepatocytes which have clear cytoplasmic vacuoles were observed respectively in the control groups. The liver is the organ involved in the metabolism, detoxification and excretion of chemicals and xenobiotics in the body (33).

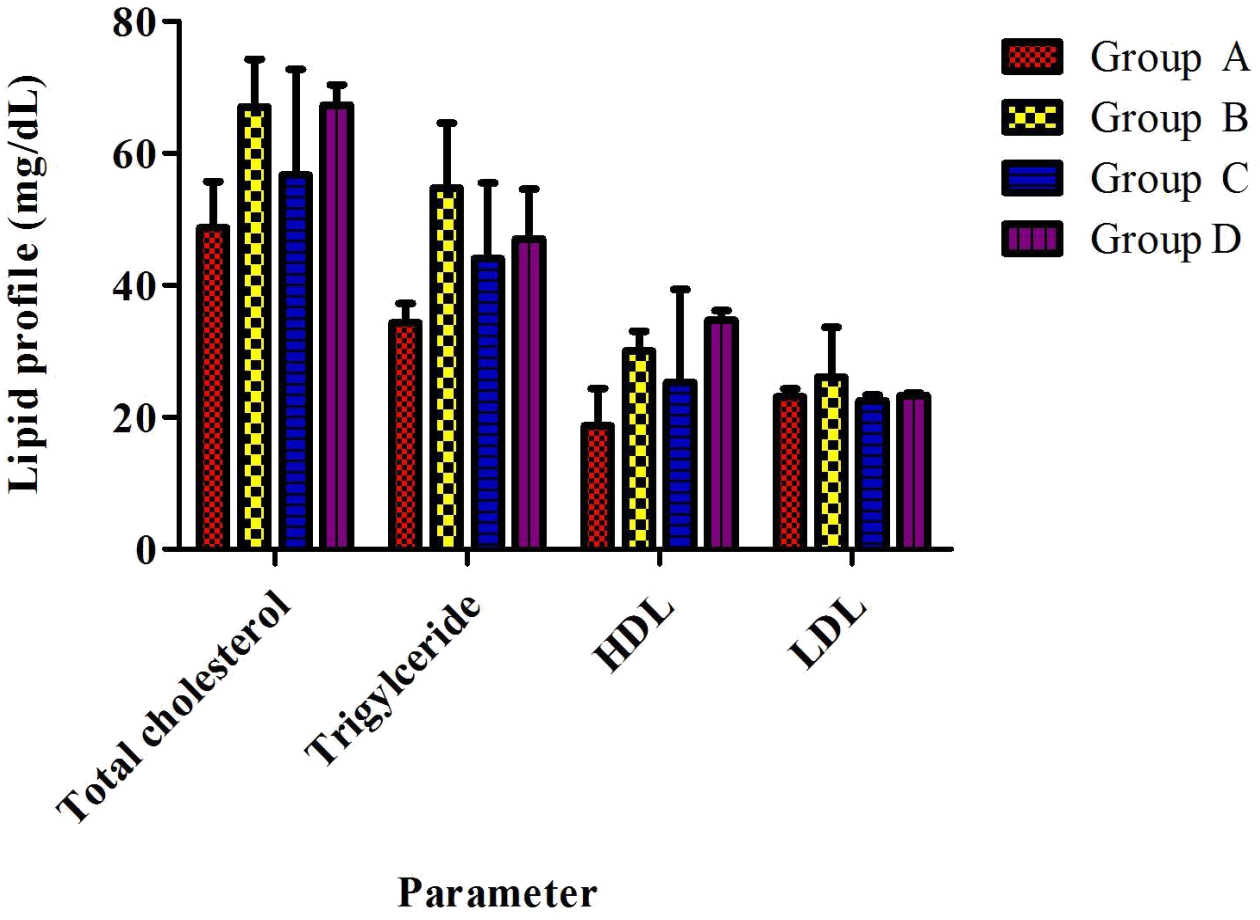

Figure 1: Graphical representation of means of total lipid profile of control and test rats

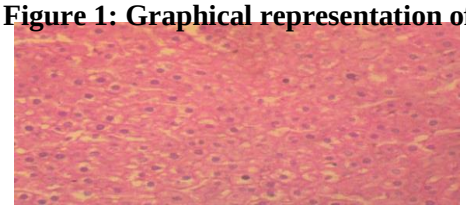

Fig. 2a: Photo micrograph of heart of rats fed with normal diet (control I) showing a few foci of mild of cardiomyocytes. H&E 400

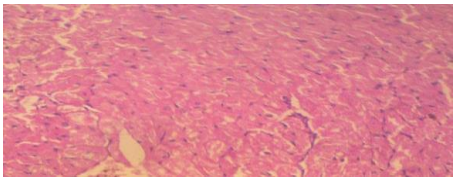

Fig. 2b: Photomicrograph of heart of rats fed with diet of 10 % groundnut oil (control II) degeneration showing a few foci of mild degeneration of cardiomyocytes. H&E x 400

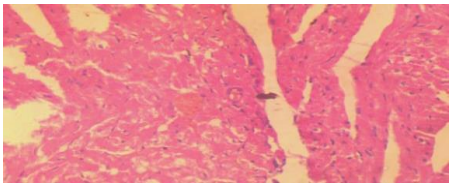

Fig. 2c: Photo micrograph of heart of rats fed with 5 % NMSO diet; cardiomyocytes appear normal.

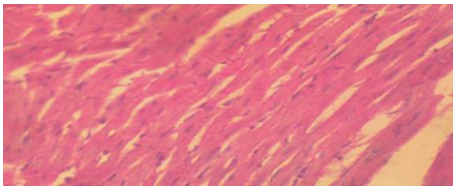

Fig. 2d: Photomicrograph of heart of rats fed with 10 % NMSO diet; cardiomyocytes appear normal

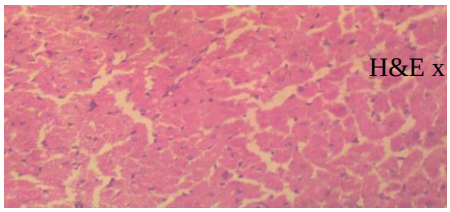

Fig. 3a: Photomicrograph of kidney of rats fed with normal fed with diet (Control I) showing mild congestion of interstitial renal blood vessels). H and E x 400

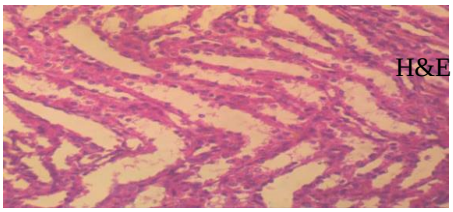

Fig. 3b: Photomicrograph of kidney of rats fed with diet of 10 % groundnut oil (Control II) showing locally extensive foci of mild sloughing off of tubular epithelium) . H & E x 400

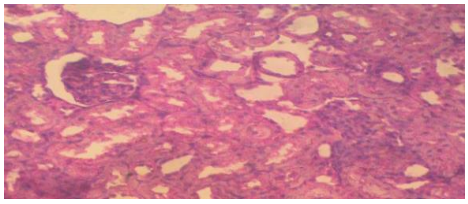

Fig. 3c: Photo micrograph of kidney of rats fed with 5% NMSO; the glomeruli, interstitum appear normal. H & E x 400

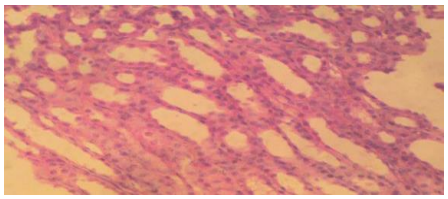

Fig. 3d: Photomicrograph of heart of rats fed with 10 % NMSO diet; there are a few tubes and renal foci of flattening of tubular epithelium. H & E x 400

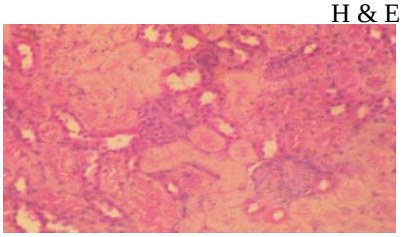

Fig. 4a: Photomicrograph of liver of rats fed with normal fed with diet (Control I) showing closely packed hepatic plates. H & E x 400

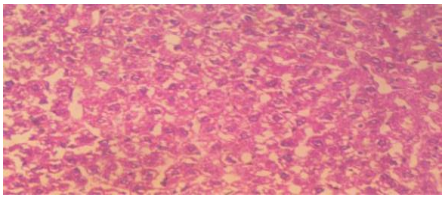

Fig. 4b: Photomicrograph of liver of rats fed with diet of 10 % groundnut oil (Control II) showing moderate thinning of hepatic plates. H & E x 400

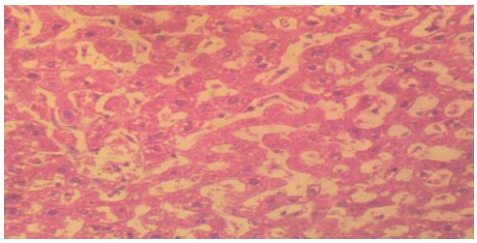

Fig. 4c: Photomicrograph of liver of rats fed with 5% NMSO diet; there are multiple thinning of hepatic plates. H&E x 400

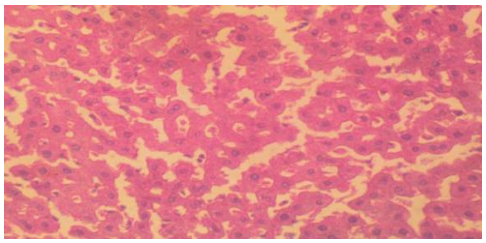

Fig. 4d: Photomicrograph of liver of rats fed with 10 % NMSO diet; there are multiple thinning of hepatic plates H&E x 400

Conclusion

N. macrophylla seed oil in moderate quantities seemed suitable for consumption and can suitably replace conventional seed oils such as groundnut oil. The seed oil can also be used in diet formulation for people with cardiovascular diseases, since it has high HDL and low LDL.

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