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Short-Term Toxicological Evaluation of *Anacardium Occidentale* Oil in Albino Rats

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Abstract
Anacardium occidentale L. nut oil was characterized after which 5% of it was incorporated in the feed formulation for albino rats. Two diets were formulated; one of these diets had 5 % *A. occidentale* oil (test diet) while the other had 5% of groundnut oil as a representative of conventional edible oil (control diet). Proximate analysis result indicated that the nut comprises of 5.82 ± 0.16% moisture, 25.54 ± 0.25% protein, 6.28 ± 0.02% crude fibre, 42.52 ± 0.45% fat, 2.90 ± 0.09% ash and 16.97 ± 0.84% carbohydrate. *A. occidentale* nut was found to be rich in potassium and magnesium. Physicochemical analysis of cashew nut oil gave 22.03 ± 0.04 mgKOH/g acid value, 22.03 ± 0.40 g/100g iodine value, 1.96 ± 0.23 mgKOH/g free fatty acid, 97.98 ± 0.99 mgKOH/g saponification value, 2.96 ± 0.59 peroxide value and 1.64 ± 0.02 refractive index. Fatty acid analysis of *A. occidentale* nut oil showed that it has high level of unsaturation (77.5754%). This is evident from the values obtained for oleic acid (61.02%), linoleic acid (15.34%) and linolenic acid (0.13%). There was weight gain by the rats during the feeding period of eight weeks and no mortality was recorded. Haematological and biochemical parameters were investigated for in the test and control rats and the results obtained revealed no adverse effect on the rats' blood. The cholesterol level in the test and control rat tissues was not significantly different at $p<0.05$. The result of the histopathology of some of the rat tissues showed no visible lesion. *A. occidentale* nut oil might be suitable for edible purposes.
Key words: *A. occidentale*, fatty acid, proximate composition, toxicology

1. Introduction

Plant seeds are important sources of oils of nutritional, industrial and pharmaceutical importance. Fatty acids are very important ingredient in human cell membranes, which affect our skin quality, resistance to disease, brain and stability of food nutritional value (USDA, 2004). Lipid composition of fruit and vegetables has lately received particular attention especially in relation to the essential fatty acids with emphasis on the health potential of polyunsaturated fatty acids (Melgarejo *et al.* 1995). Oleic acid is very important in nervous cell construction; it has fundamental role in cardiovascular disease prevention (Nasri *et al*. 2005). The high content of monounsaturated fatty acids (MUFA), especially oleic acid, is associated with low incidence of coronary heart disease (CHD) because it decreases total cholesterol and low-density lipoprotein cholesterol (Dennys *et al.* 2006). Achu *et al.* (2008) reports that linoleic acid moderately reduces serum cholesterol and LDL levels.

Anacardium occidentale, also known as cashew, is a tree in the family of the flowering plant Anacardiaceae. It is a multipurpose tree of the Amazon that grows up in warm and humid climate to 15m high. The leaves are green and have a leathery oval shape (Garcia *et al*., 2000). There are two main types; the usual cashew tree (*A. occidentale*) and *Var. nanum* (the dwarf cashew tree) (Santol *et al.,* 2007). *A. occidentale* is native to Brazil and is very common in many parts of West Africa, especially in the middle belt of Nigeria. The three main cashew products traded in international market are: raw cashew nut, cashew kernel and cashew nut shell liquid (Azam-Alli and Judge, 2001). In the industry, the fruit extract of cashew is used in body-base products. The fruit is eaten to treat scurvy, diarrhea and cholera and it is a remedy for neurological pain and rheumatic fever. It has been utilized in the treatment of premature aging of the skin and production of shampoos, lotions, and scalp creams. *A. occidentale* nut oil is used in plastic and resin industries (Achal, 2002). In traditional medicine, the extract from *A. occidentale* root, stem and fruit has been used for the treatment of dysentery, diarrhea, skin infection, fever, pain, swelling (Rajesh *et al.,* 2009) and to control arthritis and other inflammatory conditions (Ojewole, 2004). In tropical medicine, *A. occidentale* nut shell liquid is used to treat leprosy, elephantiasis, psoriasis, ringworm, diabetes and warts (Meylan *et al.,* 1999). The nuts have antioxidant, anti-cardiovascular, anti-diabetics, anti-ulcer and anti-tumor properties (Barcelos *et al*., 2007; Maylan *et al.,* 1999 and Olatunji *et al.,* 2005).

The composition of *A. occidentale* cashew have been reported by other researchers (Aletor *et al*., 2007; Arubi *et al*., 2009 and USDA, 2004) but there is no report in literature on the short-term toxicological evaluation of *A. occidentale* nut oil. This study was therefore carried out to investigate the toxicological effect of *A. occidentale* nut oil on albino rats in order to determine the possibility of replacing the conventional oils with *A. occidentale* nut oil. This is in continuation of previous work on seed oils and their nutritional /industrial applications (Ajayi

et al., 2007; 2008 and 2012).

1.1 Materials and methods

1.1.1 Sample collection and preparation

Anacardium occidentale seeds were collected from Eruwa town, Oyo State, Nigeria. These seeds were opened manually to separate the nuts from the shells. The nuts were air dried for one week under shed at room temperature after which they were reduced in size using a previously washed Philips kitchen blender (model HR 1721). The pulverized nuts were then stored prior to extraction.

1.1.2 Proximate composition

A. occidentale nuts were analyzed for ash (Pearson, 1981), fat and crude fibre (AOAC, 1995) crude protein, moisture and carbohydrate (Oyeleke, 1984).

1.1.3 Mineral composition of *A. occidentale* nut

Mineral elements in *A. occidentale* nut were determined after wet digestion of the nut with a mixture of nitric, sulphuric and hydrochloric acid using atomic absorption spectrophotometer (Perkin-Elmer, Model 2380, USA) as outlined by AOAC. (1996).

1.1.4 Physicochemical characteristics of *A. occidentale* nut oil

A. occidentale nut oil was extracted using the continuous soxhlet extraction method with n-hexane as solvent (bpt. 60^0C) for 8 h. The oil was then subjected to physical and chemical characterization. Colour and state of the oil was by visual inspection. The refractive index of the oil was determined at 29^oC using Abbe refractometer as outlined by Ajayi *et al*. (2007). The saponification, acid, free fatty acid (as oleic acid), iodine and peroxide values of the *A. occidentale* nut oil were as described by AOAC (1996).

1.1.5 Analysis of the fatty acid of groundnut and *A. occidentale* nut oils

Fatty acid composition of the oils was analyzed as their methyl esters which were prepared by boron-trifluoride methanol complex and hexane method (Official Method of Analysis, 1997). Varian 450 – gas chromatography equipped with Varian 240 flame ionization detector was used to determine the fatty acid methyl esters. Hydrogen carrier gas was used at a flow rate of 30 mL/min. Fatty acids were separated on a 1.8 x 1/8 internal detector glass column packed with 6 % BDS (butanediol succinate polyesters) on solid support Anakorm ABS (100/130) mesh. Analysis was carried out at isothermal column temperature (195^oC). Injector and detector temperatures for the analysis were at 230^oC. Gas chromatographic peaks were identified by comparison with standard methyl esters with respect to retention time and by plotting the log of retention times against equivalent carbon length (ECL). Peaks were measured by Varian electronic integrator. The percentage of each peak was calculated as the percentage of the total area of all the peaks.

1.1.6 Experimental animals

Fourteen weanling albino rats (aged 6 weeks, weighing between 61-76 g) were obtained from the Animal Experimental Unit, Veterinary Department, University of Ibadan, Ibadan, Nigeria.

1.1.7 Feed compoundment and animal grouping

Feed was formulated to meet the entire nutrient requirement for young rats. The feed was prepared according to the method of Toyomizu *et al*. (2003) with slight modification. The basic ingredients used were: maize (40%), soy bean (18.21%), groundnut cake (9.45%), *A. occidentale* nut oil (5%), bone (3.30%), salt (0.79%), palm kernel (0.7%), wheat (0.7%), corn bran (0.7%) and limestone (2.26%). The control feed had 5% of groundnut oil incorporated into it while the experimental feed contained 5% inclusion of *A. occidentale* nut oil as a total replacement for groundnut oil. Ingredients of feeds were mixed thoroughly by a mixing machine, pelletized and dried. The animals were completely randomized into two groups of seven rats each. Animals in the control group were maintained on groundnut oil-based feed while those in experimental group were fed on *A. occidentale* nut oil-based feed. The rats were fasted for 5 h before the commencement of the experiment and were thereafter fed on their respective diet for 8 weeks. The amount of feed consumed was noted daily and computed on weekly basis. Weight of the rats was recorded on weekly basis for the 8 weeks feeding period. The animals were handled according to the guidelines of the Ethical Committee on the Use and Care of Experimental Animals Unit of the Department of Veterinary Medicine, University of Ibadan, Nigeria.

1.1.8 Blood sample and tissue collection

At the end of the feeding period of eight weeks, the rats were fasted overnight and blood sample was collected through ocular puncture into sample bottles containing EDTA to prevent blood coagulation. In one part of the blood, the haematological studies was carried out and to the other part, plasma was separated by centrifugation. In the blood plasma the total cholesterol was determined. The animals were sacrificed through cervical dislocation method (Klaung *et al*., 2004) and dissected to remove the internal organs. The tissues collected were kidney, heart, spleen, lungs, small intestine, brain and liver. These organs were weighed immediately after collection and cut into two; one-half was preserved in containers containing 10% formalin for pathological

studies. The photomicrograph slide of the organs were prepared and observed (Ajayi *et al*., 2007).

1.1.9 Total cholesterol, haematology and biochemical parameters determination

The white blood cell (WBC) and the red blood cell (RBC) were determined using Neubaurer haemocytometer. Packed cell volume (PCV) was determined by microhaematocrit centrifuge. Mean corpuscular haemoglobin (MCH) and Mean corpuscular haemoglobin concentration (MCHC) were determined according to the method of Jain (1986). Analysis was also carried out for Total protein (TP), Albumin (ALB) and Globulin (GLB). Total **c**holesterol was determined according to Fredrickson *et al*. (1986).

1.1.10 Statistical Analysis

Means were analyzed using a one-way analysis of variance (ANOVA) and complemented with Student's t-test. Differences with values of $P < 0.05$ were considered statistically significant (Mahajan, 1997).

1.2 Results and discussion

1.2.1 Proximate composition

The protein (25.54 ± 0.25%) and fat content (42.52 ± 0.45%) of *A. occidentale* nut were relatively high (Table 1). This protein content is higher than those of high protein animals such as lamb, marine fishes and beef, 16.0-18.0 g/100 g dry matter (Bhuiyan *et al*., 1986) but it is close to that of *Ganavalia ensiforms* which is an underutilized legume (Ajah and Madubuike, 1997). It suggests that the nut can contribute to the daily protein need of 23.6 g/100g for adults as recommended by the National Research Council (1974). The oil yield of the nut is higher than those reported for various soybean cultivars 18.30-21.53 g/100 g dry matter (Vasconcelos *et al*., 1997).

1.2.2 Mineral element analysis

The result of the mineral analysis of the nut is shown on table 2. It reveals that potassium had highest concentration; this is in line with what has been reported by Kimbonguila *et al.* (2009). *A. occidentale* nut is rich in calcium (153.6 ± 0.53 mg/kg) and magnesium (5565 ± 0.58 mg/kg). These values are in line with the value reported for cashew kernel (Omoje *et al.*, 2008). Calcium is responsible for bone formation, while magnesium is responsible for maintaining body metabolism and transition of nerve impulse. Ca or Mg plays a significant role in photosynthesis (Aremu *et al.*, 2005).

1.1.3 Physicochemical parameters

Even though there were significant differences between the physicochemical characteristics of *A. occidentale* nut oil and groundnut oil (Table 3), the characteristics of the nut oil of *A. occidentale* showed that the oil may be a good replacement for conventional edible oil like groundnut oil (Oladiji *et al*., 2007).

1.1.4 Fatty acid composition

The percentage composition of the fatty acid present in *A. occidentales* nut oil showed that the oil is highly unsaturated (77.5754%); it is much higher that of the groundnut oil (59.8245%) that was used as the control oil (Table 4). The main saturated fatty acids were palmitic (13.87%) and stearic (7.38%) acids while the main unsaturated fatty acids were oleic (61.01%), linoleic (15.35%) and linolenic (0.13%). The linoleic acid content of *A. occidentale* nut oil (15.34%) is higher than that of groundnut oil (12.94%). The oleic acid is also higher; 61.01% for *A. occidentale* nut oil compared to 45.55% for groundnut oil. Achu *et al.* (2008) reports that linoleic acid moderately reduces serum cholesterol and LDL levels. It has also been reported by El-Adawy (2001) that the presence of high amounts of essential linoleic acid in the oils studied suggests that the oils are highly nutritious due to their ability to reduce serum cholesterol. Oleic acid is very important in nervous cell construction; it has fundamental role in cardiovascular disease prevention (Nasri *et al*., 2005). The high content of monounsaturated fatty acids (MUFA), especially oleic acid, is associated with low incidence of coronary heart disease (CHD) because it decreases total cholesterol and low-density lipoprotein cholesterol (Dennys *et al.*, 2006). A rapidly growing literature illustrates the benefits of PUFA in alleviating cardiovascular, inflammatory conditions, heart disease, atherosclerosis, autoimmune disorders, and diabetes (Finsley and Shahidi 2001; Riemersma 2001, Ramadan *et al.* 2006). Both oils contain some amount of linolenic acid; 0.1255% and 0.3947% for *A. occidentale* oil and groundnut oil respectively.

1.1.5 Body and Feed weight

There were steady increments in the body weight and the feed intake per week of rats in both groups within the period of this experiment (Tables 5). In the control group, the weight of the rats increased from 70.13 ± 6.08 g to 192.33 ± 33.56 g while in the test group, it increased from 66.99 ± 3.96 g to 179.11 ± 26.66 g. This increase in weight is an indication that 5% inclusion of both *A. occidentale* nut and groundnut oils in the rat feed supported growth. Feed consumption increased from 691.85 g to 7027.91 g and 603.62 g to 7236.04 g for the control and test groups respectively. The increased feed consumption by rats implies that the compounded feeds enhanced the sense of taste and appetite of the animals after consumption (Marai *et al*., 1996). This promoted the growth of the rats. In addition, no mortality was recorded in both groups (control and test) during the feeding period.

1.1.6 Organ weight

Table 6 shows the organ weights of test and control rats at the end of eight weeks of the experiment. The organs harvested and weighed were liver, kidney, lung, heart, brain, spleen and intestine. The weight of heart, brain, lungs, kidney, liver and intestine of group A rats were 0.67 ± 0.08, 1.47 ± 0.13, 1.46 ± 0.25, 1.23 ± 0.02, 7.13 ± 1.39, and1.4 ± 0.34 g respectively. Similar weights were recorded for rats in group B. Similarly, there were no significant differences between different organs of the control and test group rats (except for that of the brain) at $p<0.05$ and there were also no overt signs of clinical toxicity.

1.1.7 Haematological and biochemical analyses

The result of haematological and biochemical analyses of blood of rats fed with 5% inclusion of *A. occidentale* nut and groundnut oils in rat feed is shown on Table 7. The concentration values reported for rats in control and test groups are comparable, though there are variations in the value of white blood cell and absolute lymphocyte. Haematological results indicate that there were no major complications in the blood of the test rats. This might have been responsible for the high survival rate observed in the rats during the experiment. The haematological and biochemical parameters in control and test groups are comparable, but there is a slight different in the value of white blood cell and absolute lymphocyte. Haemoglobin concentration of the test group is slightly different from that of the control group. Ajayi *et al.* (2007) reported a lower haemoglobin concentration for rats fed with *G. mangostana* seeds oil. Mean corpuscular volume, mean corpuscular haemoglobin volume and total protein concentration are significantly different in the two groups ($p < 0.05$). Percentage lymphocyte, neutrophyl, eosinophil and monocyte of the blood of the rats in the two groups compared favourably. The values for biochemical analysis such as total protein, albumin, globulin and albumin/globulin ratio obtained for both groups are similar. This is an indication that *A. occidentale* nut oil probably had no significant adverse effect on the blood of test rats.

1.1.8 Histological analyses

The heart and spleen of the test rats showed no visible lesion (Figs. 1 and 4). However the brain of one the test rats showed visible necrosis congested blood vessels (Fig. 2); the stomach of a rat in test group also showed the presence of a flat worm (Fig. 3).

Conclusion

A. occidentale nut is high in crude protein, carbohydrate and crude fibre and could therefore find application in feed formulation. It is rich in potassium, which is beneficial in protein synthesis and magnesium. The iodine value of cashew nut oil placed it in the non-drying group of oil. Vital organs of test rats did not show severe lesions but compared well with those of the control rats. The same trend was observed in the haematological and biochemical result of the blood samples of rats from both groups. *Anacardium occidentale* nut oil could probably be a suitable replacement for conventional seed oils.

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Table 1: Proximate analysis of *A. occidentale* nut

Parameter (%)	Mean ± SD
Moisture content	5.82 ± 0.16
Crude protein	25.54 ± 0.25
Crude fibre	6.28 ± 0.02
Fat content	42.52 ± 0.45
Ash content	2.90 ± 0.09
Carbohydrate	16.97 ± 0.84

Values are expressed as mean ± SD for 3 results

Table 2: Mineral composition of *A. occidentale* nut (mg/kg)

Metals	Mean ± SD
Calcium	153.6 ± 0.53
Magnesium	5565 ± 0.58
Potassium	15800 ± 0.00
Sodium	331 ± 1.00
Manganese	16.03 ± 0.06
Iron	171.66 ± 0.58
Copper	2.13 ± 0.06
Zinc	93.3 ± 0.17

Table 3: Physicochemical analysis of groundnut and *A. occidentale* nut oils

Parameter	**Groundnut oil**	***A. occidentale* nut oil**
Acid value (mgKOH/g)	9.21 ± 0.72^{a}	22.03 ± 0.40^{b}
% Free fatty acid	4.63 ± 0.36^{a}	1.96 ± 0.23^{b}
Saponification value (mgKOH/g)	72.09 ± 0.74^{a}	97.78 ± 0.99^{b}
Iodine value (g/100g)	11.32 ± 0.66^{a}	22.03 ± 0.40^{b}
Peroxide value	2.28 ± 0.28^{a}	2.96 ± 0.59^{a}
Refractive index	1.42 ± 0.05^{a}	1.64 ± 0.02^{b}

Values along the same row having different superscript are significantly different ($p < 0.05$)

Table 4: Result of fatty acid composition of groundnut and *A. occidentale* nut oils (%)

Fatty acid	**Groundnut oil**	***A. occidentale* nut oil**
C12 : 0	0.4883	-
C14 : 0	0.9658	-
C16 : 0	31.9979^{a}	13.8678^{a}
C17 : 0	-	0.1433
C17: 1	-	0.0578
C18 : 0	3.9404^{a}	7.3794^{b}
C18 : 1 (n 9)	45.547^{a}	61.0150^{b}
C18 : 2 (n 6)	12.9407^{a}	15.3388^{a}
C18 : 3 (n3)	0.3947^{a}	0.1255^{a}
C20 : 0	0.3947^{a}	0.1487^{a}
C20 : 3 n 6	0.2981^{a}	0.3614^{a}
C22 : 1 n 9	0.1949^{a}	0.2278^{a}
C22 : 6 (n3)	0.3527^{a}	0.3453^{a}
C24 : 0	0.1974^{a}	0.1325^{a}
C24 : 1	0.0964^{a}	0.1038^{a}
Unknown	2.1910	0.7529
SFAs	37.9845	21.6717
UFSAs	59.8245	77.5754

Values along the same row carrying different superscript are significantly different ($p < 0.05$)
SFAs= Saturated fatty acids; UFSAs= Unsaturated fatty acids

Table 5: Result of weight increase of rats per week (g)

Week	Control group	Test group
0	70.13 ± 6.08[a]	66.99 ± 3.96[b]
1	88.99 ± 7.27[a]	89.68 ± 9.45[b]
2	108.54 ± 11.94[a]	108.07 ± 11.96[b]
3	127.06 ± 16.80[a]	126.24 ± 13.66[b]
4	146.56 ± 22.43[a]	142.71 ± 15.19[b]
5	158.06 ± 25.14[a]	150.18 ± 15.70[b]
6	173.49 ± 28.45[a]	158.89 ± 19.8 [b]
7	182.89 ± 30.89[a]	168.96 ± 22.80[b]
8	192.33 ± 33.56[a]	179.11 ± 26.66[b]

Mean with the same alphabets are not significantly different at $p < 0.05$
Values are expressed as mean ±SD for weights of seven rats in each group per week

Table 6: Result of weight of tissues (g)

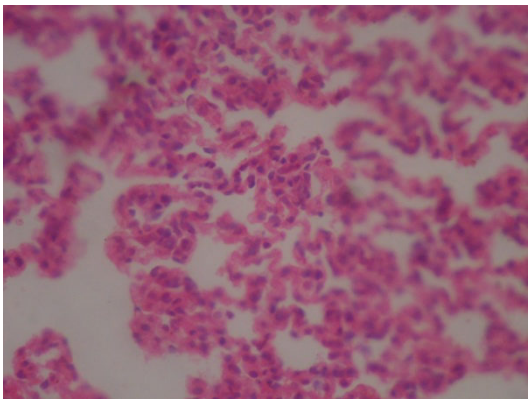
Tissue	Control group	Test group
Liver	7.13 ± 1.39[a]	6.74 ± 1.40[a]
Kidney	1.23 ± 0.20[a]	1.11 ± 0.18[a]
Heart	0.67 ± 0.08[a]	0.61 ± 0.09[a]
Lungs	1.46 ± 0.25[a]	1.46 ± 0.22[a]
Intestine	1.40 ± 0.34[a]	1.3 ± 0.22[a]
Spleen	0.74 ± 0.26[a]	0.57 ± 0.14[a]
Brain	1.47 ± 0.13[a]	1.30 ± 0.06[a]

Mean with the same alphabets are not significantly different at $p < 0.05$
Values are expressed in means± SD (n=7) for the two groups

Table 7: Results of haematological and blood biochemical analysis

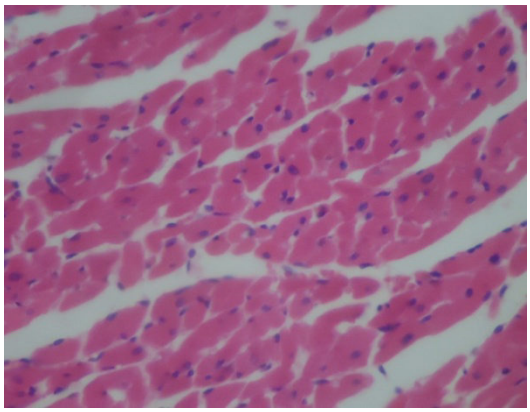
Parameter	Control group	Test group
PVC (%)	32.00 ± 1.73[a]	32.86 ± 1.57[a]
HB (mg/dl)	10.47 ± 0.63[a]	10.61 ± 0.48[a]
RBC (10^6/µl)	4.88 ± 0.24[a]	5.19 ± 0.45[a]
WBC (10^3/µl)	6642.86 ± 2028.22[a]	7864.29 ± 3010.22[a]
MCV (fl)	66.24 ± 1.76[a]	58.40 ± 8.63[b]
MCHC (%)	32.72 ± 0.71[a]	32.31 ± 0.36[a]
MCH	21.44 ± 0.47[a]	20.52 ± 1.00[b]
Lymphocyte (%)	1.22 ± 0.65[a]	0.98 ±0.24[a]
Neutrophyl (%)	0.46 ± 0.35[a]	0.36 ± 0.17[a]
Eosinophyl (%)	0.02 ± 0.02[a]	0.01 ± 0.02[a]
Monocyte (%)	0.05 ± 0.03[a]	0.04 ± 0.02[a]
Absolute lymphocyte	70.71 ± 5.85[a]	73.43 ± 7.91[a]
Absolute neutrophyl	24.86 ± 6.26[a]	25.14 ± 6.84[a]
Absolute eosinophyl	1.14 ± 1.68[a]	0.57 ± 0.98[a]
Absolute monocyte	2.71 ± 0.49[a]	2.71 ± 1.25[a]
Platelets	120857.14 ± 8610.62[a]	151714.29 ± 65152.93[a]
Total Protein (g/dl)	8.63 ± 0.16[a]	8.77 ± 0.05[b]
Albumin (g/dl)	5.14 ± 0.15[a]	5.20 ± 0.12[a]
Globulin (g/dl)	3.50 ± 0.12[a]	3.56 ± 0.15[a]
A.G Ratio	0.61 ± 0.07[a]	0.64 ± 0.08[a]
Cholesterol (mg/dl)	67.33 ± 6.24[a]	81.33 ± 7.02[a]

Mean with different alphabets are significantly different at $p < 0.05$
Values are expressed in means± SD (n=7) for the two groups



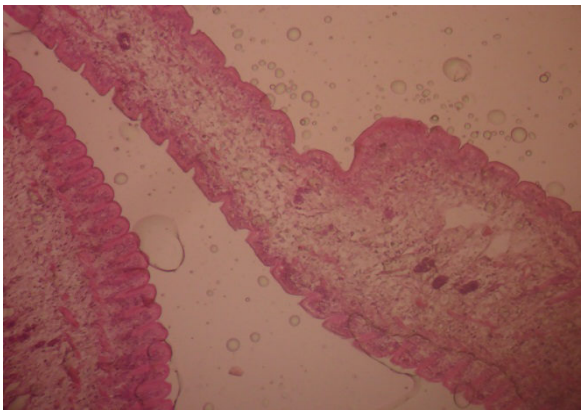
H x E x400

Fig 1. Photomicrograph of the heart of rat given 5% of cashew nut oil showing no visible lesion



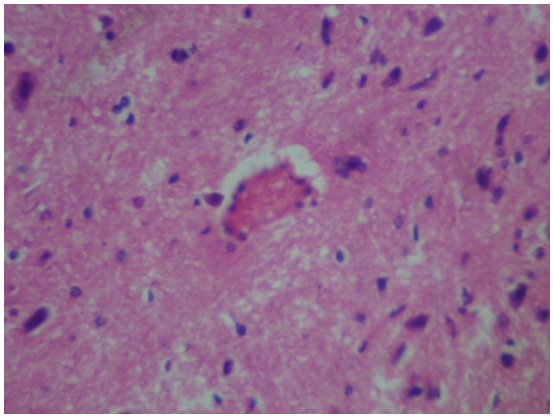
H x E x400

Fig 2. Photomicrograph of the brain of rat given 5% of cashew nut oil showing neuronal necrosis congested blood vessels



H x E x400

Fig 3. Photomicrograph of the stomach of rat 5% of cashew nut oil showing lumen containing a flatworm



H x E x400

Fig 4. Photomicrograph of the spleen of given rat given 5% of cashew nut oil showing no visible lesion