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Full Length Research Article

Haematological and Hepatho-Renal Effect of Cashew Nut Oil on Male Wistar Rats

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ABSTRACT
The aim of this study was to evaluate the biosafety of *Anacardium occidentale* (cashew) nut oil using male Wistar rats as model. Cashew nut oil was extracted from cashew nuts with the aid of a Soxhlet apparatus using n-hexane as solvent. Twelve male Wistar rats were randomly assigned to four groups labeled I, II, III and IV (3 rats per group). Group I served as the control group and were administered distilled water. Group II received 1.53 mL/kg of the nut oil; group III received 4 mL/kg of the nut oil while group IV received 8.13 mL/kg of the nut oil. The administrations were via the oral route. Blood samples were collected weekly for haematological examination. At the end of four weeks study period, the rats were anaesthetized and the liver and kidneys were removed for histological examination. The haematological result showed slight deviation from the normal in packed cell volume (52%), total white blood cell count (9.38 x 10^3/mm^3) and lymphocyte count (58.17%) in Wistar rats administered 4 to 8.13 mL/kg of the cashew nut oil, respectively yet no visible compromise of the immune system was observed. Histological analysis; however, revealed some hepatocyte morphological lesions including mild microvesicular steatosis, kupffer cell hyperplasia, shrinkage of glomeruli, glomerular distortion, necrotic renal tubules, severe congestion and atrophy of renal tubules in rats fed 8.13mL/kg of the nut oil. In conclusion, the cashew nut oil was responsible for hepatic parenchymal and matrix alteration in wistar rats administered 8.13 mL/kg of cashew nut oil.
Keywords: Cashew nut oil, Haematological parameters, Histopathology, Liver, Kidney and Wistar rats

INTRODUCTION
Many countries, especially in the tropics are involved in the production of cashew for a variety of food and industrial products (Menezes and Alves, 1995). According to FAO (2010), Vietnam, Nigeria, India, Indonesia and Brazil are the world's leading producers of cashew nuts. The cashew apple and the nuts are the edible parts of *Anacardium occidentale* which may be consumed raw or process into other consumable products (Duke, 1983). However, cashew nuts cause allergic reactions that range from mild discomfort even to life-threating anaphylaxis (Weinberger and Sicherer, 2018).

Cashew nut oil has been reported to have strong repellent effect on the larvae of *Dermestes maculatus* (Akpotu and Adebote, 2013). Ileke and Olutuah (2012) also reported that cashew seed powder and oil resulted in 63.3 to 100% mortality of *Callosobrochus maculatus*. According to Nwaogu *et al.* (2013), 0.5ml, 1ml and 1.5ml cashew nut oil treatments resulted in 100% mortality of *C. maculatus*. Cashew nut oil has also found potential use as a preservative in smoke-dried fish storage, both as a protectant and in lengthening fish shelf-life (Akpotu *et al*., 2021). Several studies have been conducted to evaluate biosafety of edible oils. A sub-acute study on black caraway seed essential oil for example revealed vacuolar degeneration, vascular congestion, atrophy of hepatocytes and dilated sinusoidal spaces in the liver as well as degenerative changes in cells of the renal tubules and atrophy of the glomeruli of the kidney in Wistar rats (Tabarraei *et al*., 2019). In another study, caraway oil obtained from the fruits of *Carum carvi* L. was shown not to induce significant changes in the haematological parameters and histopathology in treated rats when compared with similar data from the control animals (Auti and Kulkarni, 2019). Tende *et al*. (2011) reported that administration of garlic and ginger as well as its combination to animals significantly alter the serum electrolytes and decreased the packed cell volume and haemoglobin concentration with an elevated red cell count of rats. At the moment, there is no information on the effect of cashew nut oil on the blood and vital organs of mammalian consumer. This study was therefore, designed to evaluate the biosafety of *Anacardium occidentale* (cashew) nut oil using male wistar rats as model.

MATERIALS AND METHODS
The experiment was conducted in the Farm house of the Department of Veterinary Pharmacology and Physiology, Ahmadu Bello University, Samaru, Zaria, Kaduna State, Nigeria at room temperature of between 25-30˚C and 56% relative humidity, within the months of May to June 2017.

The nut oil of Cashew (*Anacardium occidentale* L.) used for this study was obtained from nuts collected from fruiting trees within the Main Campus of Ahmadu Bello University, Samaru, Zaria. The Cashew plant was properly identified and authenticated at the Herbarium unit, Department of Botany, Ahmadu Bello University, Zaria, prior to processing. Cashew nuts were removed from its respective pods then sun-dried for 10 days after which it was pulverized using a mortar and pestle. The pulverized seeds were sieved and stored in pre – labeled cellophane bags. The seed powder was weighed (40 g) into separate muslin cloth and introduced separately into the Soxhlet chamber for the oil

extraction with n – hexane as extraction solvent. The extraction was done at 60 - 80˚C until the solvent in the Soxhlet chamber became transparent. The extracted oil was concentrated with a Rotatory evaporator at 60 – 80˚C to recover solvent free oil, stored in a labeled bottle and kept in a cool dry place until used for bioassay.

The total oxalates were determined by adopting the procedure described by Fasset (1996). Trypsin inhibitor activators of cashew nut oil were determined as described by Liener(1979). Tannins content was determined by the method described by Jaffe (2003) while phytate content was determined by the procedure described by Lucas and Markakas (1975).

Experimental Design and Treatment of Animals

Adult male albino rats weighing between 150-170 g housed under standard laboratory conditions with a 12 hours daylight cycle and had free access to feed and water were allowed to acclimatize to laboratory conditions for 14 days before the commencement of the experiments, which were carried out in compliance with the recommendations of Helsinki's declaration (2016) on guiding principles on care and use of animals. Ethical clearance was sought from the Ahmadu Bello University Committee for Animal Use and Care with Approval number ABUCAUC/2018/005.

The rats were randomly assigned into four groups (I, II, III and IV). Group I served as the control and II-IV as test groups. Groups II, III and IV were oral-gavaged daily with 1.53 mL/kg, 4mL/kg and 8.13 mL/kg of the oil respectively while Group I was served water. The rats were fed with commercial grower feed and allowed free access to water. Blood samples were collected weekly through the retro-orbital route for haematological analysis.

At the end of week four, the experimental animals were weighed and then sacrificed under chloroform anaesthesia. The liver of the rats was collected and placed in EDTA bottles containing Bouin's fluid (for tissue preservation) and then taken to the Histopathology Laboratory, Ahmadu Bello University Teaching Hospital, Zaria for histopathological examination.

Determination of Haematological Parameters

The anti-coagulated blood was used for the determination of haematological indices. The red blood cells (RBC) and white blood cells (WBC) counts were determined by the improved Neubauer haemocytometer method. The haemoglobin (Hb) concentration was determined according to Jain (1986), using the cyanomethaemoglobin method. The packed cell volume (PCV) was determined by the micro-haematocrit method described by Dacie and Lewis (1991). Schilling method of differential lecukocyte count was used to determine the distribution of the various white blood cells (Mitruka and Rawnsley, 1977).

Histological Procedure

The harvested liver and kidney tissues from all the experimental groups were sectioned fixed in Bouins fluid and processed using routine histological technique and embedded in paraffin wax. Sections were cut at the thickness of 5 microns and stained with haematoxylin and eosin stain (Drury and Wallington, 1976). The hepatic lesions were identified according to the following criteria: (I) hydropic degeneration, (II) microvesicular steatosis, (III) necrosis (hypostained or absent nucleus, intense cytoplasmic eosinophilia, and destruction or loss of the architecture of the hepatocyte cord), and (IV) apoptosis.

Statistical Analysis

A One-Way Analysis of Variance (ANOVA) at $p<0.05$ was used to determine if significant difference exist between the various blood parameters. Tukey's Test was used to separate the means.

RESULTS

The cashew nut oil was screened for the following Antinutrients: Oxalate, Tannins, Phytate, Alkaloid and Trypsin inhibitor. They were present in 0.003 mg/100g, 0.5 mg/100g, 3.26 mg/g, 1.13% and 0.31 mg/g respectively which fell within the acceptable range given for edible food (Table 1).

Table 2 shows the result of the response of blood parameters to the different doses of *A. occidentale* administered to Wistar rats. The mean values of the Packed Cell Volume (PCV) were higher in the test groups in comparison to the control group. Rats in Group I had the highest PCV values (52%) while the control group had the least PCV values (42.25%). The haemoglobin content (HGB) and total protein (TP) were highest (17.32 and 7.19 g/dl, respectively) in group III compared to the control group (14.19 and 6.52 g/dl). Also, Total Red Blood Cell (TRBC) and Lymphocytes counts were highest (9.31 and 58.17%, respectively) in group III in comparison to the control which had the least (7.28 and 54%). However, the mean Total White Blood Cell (TWBC) and the Neutrophil (NEUTRO) contents were highest (12.32 $x10^3/mm^3$ and 42.92%, respectively) in the control group and lowest in the test groups (9.38 $x10^3/mm^3$ and 39.5%). Monocytes and eosinophils of experimental groups I and II were higher (1.42 and 0.84%, respectively) than that of the control group (0.92 and 0%). The values of all these parameters fell within the acceptable normal reference ranges for Wistar rats.

Table 1: Antinutrients content of *A. occidentale*

ANTINUTRIENTS	OXALATE (MG/100G)	TANNINS (MG/100G)	PHYTATE (MG/G)	ALKALOIDS (%)	TRYPSIN (MG/G)
Mean value	0.003±0.00	0.5±0.003	3.26±0.06	1.13±0.05	0.31±0.006
FAO Standard (2009)	< 3.00	< 3.00	>15.00	<3.00	<5.00

Mean ± SEM along columns is not significantly different at P < 0.05

Table 2: Haemathological components responses of male wistar rats after ingestion of *Anacardium occidentale* nut oil

BLOOD PARAMETERS	CONTROL GROUP	GROUP I (1.53 mL/kg)	GROUP II (4mL/kg)	GROUP III (8.13 mL/kg)	REFERENCE RANGE (≤ 6 MONTHS)	p VALUE
PCV (%)	42.25±1.48^{a}	52±1.09^{b}	51.92±1.77^{b}	50.67±1.59^{b}	36.4 - 41.8	0.005
HGB (g/dl)	14.19±0.56^{a}	17.29±0.36^{b}	17.21±0.06^{b}	17.32±0.41^{b}	12 - 14	0.001
TP (g/dl)	6.52±0.19	6.72±0.12	6.62±0.16	7.19±0.34	6.56 - 7.64	0.218
TWBC (x10^{3}/mm^{3})	12.32±1.21	11.22±0.62	12.81±0.61	9.38±0.72	6.89 - 12.91	0.075
TRBC (x10^{6}/mm^{3})	7.28±0.31^{a}	8.49±0.20^{ab}	8.72±0.35^{ab}	9.31±0.42^{b}	6 - 8	0.014
NEUTRO (%)	42.92±2.54	37.83±1.24	41.25±1.05	39.5±0.36	10 - 46	0.179
LYMPHO (%)	54±3.04	58.58±1.42	56.25±1.32	58.17±0.36	45 - 85	0.329
MONO (%)	0.92±0.21	1.42±0.51	1.42±0.32	0.67±0.14	2 - 6	0.337
EOSINO (%)	0±0^{a}	0.84±0.24^{ab}	0.08±0.05^{ab}	0.42±0.24^{b}	0 - 6	0.032

PCV=Packed Cell Volume, HGB=Haemoglobin content, TP=Total Protein, TWBC=Total White Blood Cell, TRBC=Total Red Blood Cell, NEUTRO=Neutrophil, LYMPHO=Lymphocyte, MONO= Monocyte, EOSINO= Eosinophil (Means±SE with the same superscript along a row are not significantly different at p<0.05) (Wolford *et al*., 1986; Filho *et al*., 2017)

The liver of rats in the control group showed normal histological parenchyma with the central vein, hepatocyte and sinusoid seen (Plate I). Each lobule showed radially arranged hepatocytes, forming cords around the central veins. Hepatocytes appeared polygonal in shape with rounded central vesicular nuclei. Blood sinusoid were seen separating cords of hepatocytes and lined by flattened endothelial cells and Kupffer cells. The histological examination of rats fed nut oil (1.53 mL/kg) showed normal Central portal vein (CV) and the hepatocytes with numerous kupffer cells (Plate II). Microscopic observations of the liver of rats fed 4mL/kg revealed slight nuclear proliferation, sinusoids raminify the liver and begin to show spaces (Plate III) while rats fed 8.13mL/kg showed abnormal hepatic cells, more pronounced dilated congested blood vessels in central vein, pyknotic cells, vacoulation and necrotic areas (Plate IV).

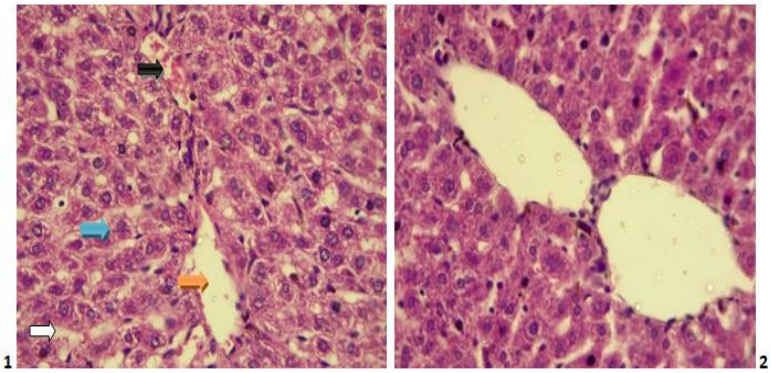

Plate I: Photomicrograph of the normal liver tissue (Control) of adult albino rat. The periportal artery (black arrow) and the hepatocytes (blue arrow) with round nucleus, normal central vein (yellow arrow) and sinusoids (white arrow) raminify the liver (H.&E., X400)

Plate II: Photomicrograph of the liver treated with Nut Oil 1.53mL/kg. The Central vein and the hepatocytes with numerous kupffer cells (black arrow) (H.&E., X400)

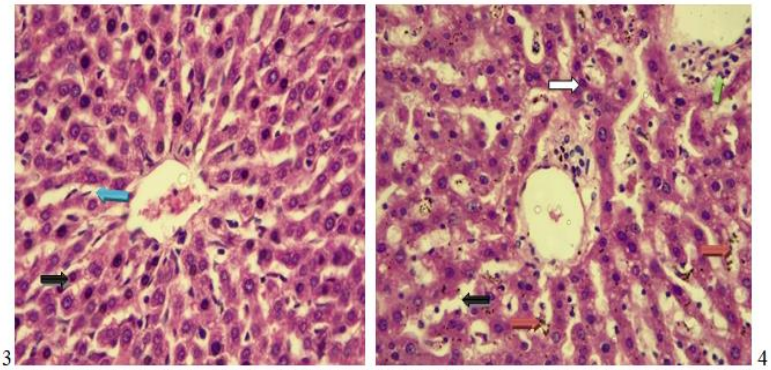

Plate III: Photomicrograraph showing the liver treated with Nut Oil 4mL/kg note the central portal vein and the hepatocytes degenaration with proliferating nuclea (black arrow), sinusoids (blue arrow) raminify the liver and begining to show spaces (H.&E., X400)

Plate IV: Photomicrograph of the liver treated with Nut Oil 8.13mL/kg showing abnormality in the structure and congestion around the central vein and the hepatocytes have pyknotic nuclei (white arrow), sinusoids raminify the liver with wider spaces (black arrow); shrinkage in the composite cells and fat droplets (Fatty liver) and accumulation of mononuclear cells in the vicinity of sinusoids (green arrow). The sinusoid walls show numerous pigmentation (red arrow) (H.&E., X400)

Effect of Cashew Nut oil on Rat kidney

Histopathological examinations of kidney sections of the control group of animals revealed the renal corpuscles were formed of lobulated glomeruli surrounded by Bowman's spaces. The proximal convoluted tubules appeared to be lined by a single layer of cuboidal epithelium enclosing a narrow lumen. The distal convoluted tubules were lined by cuboidal cells surrounding wider lumen (Plate V). Rat fed nut oil 1.53 mL/kg did not reveal any shrinkage with lobulation or fragmentation of glomerular apparatus, but increase in Bowman's space was beginning to set in (Plate VI). Histopathological examination of rat fed with nut oil 4mL/kg showed moderate pathological effect represented by shrinkage with lobulation or fragmentation in glomeruli leading to increase of Bowman's space, but the most histological structures of the kidney are still normal as in the control group (Plate VII) while the rats fed nut oil 8.13mL/kg showed obvious damage of renal tubules, as shrinkage with glomerular distortion, and atrophy of renal tubules with multi necrotic tubular cells, or parenchymatous degeneration of cells of renal tubules (Plate VIII).

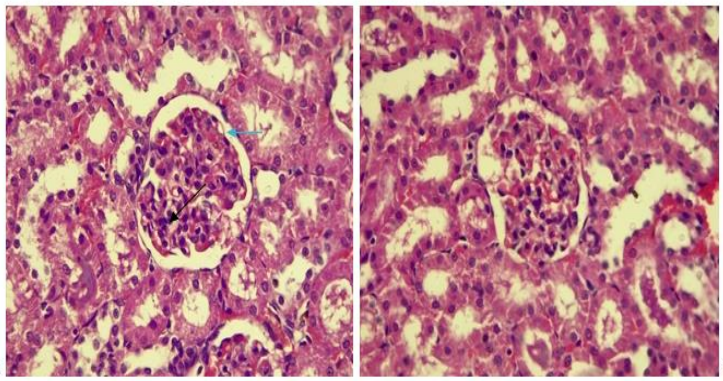

Plate V: Photomicrograph of normal Kidney structure of adult Wistar rat (Control) with normal Bowman's capsule (blue arrow) and Glomerular apparatus (black arrow) (H.&E., X40)

Plate VI: Photmicrograph of kidney of wistar rat treated with Nut Oil 1.53mL/kg: Shrinkage with lobulation or fragmentation of glomerular apparatus not observed (H.&E., X400)

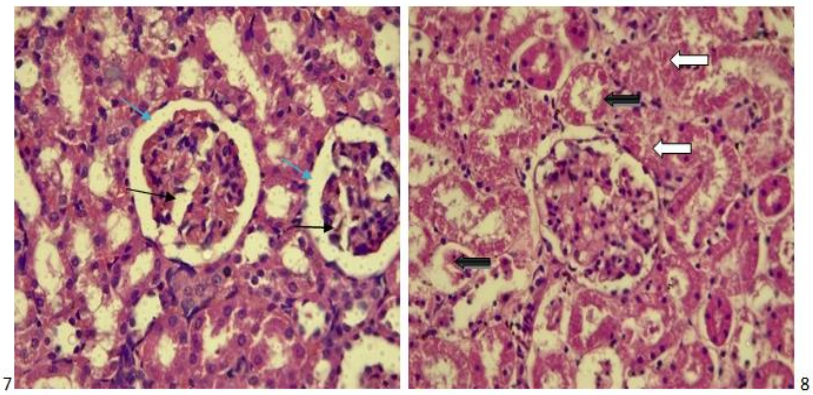

Plate VII: Photomicrograph of Kidney of wistar rat treated with Nut Oil 4mL/kg showing moderate toxicological effect represented by shrinkage with lobulation or fragmentation (black arrow) in glomeruli leading to increase of Bowman's space (blue arrow) but the most histological structure of the kidney still normal like in the control group(H.&E., X400)

Plate VIII: Photomicrograph of kidney treated with Nut Oil 8.13mL/kg showing obvious damage of renal tubules, as shrinkage with glomerular distortion (white arrow), and atrophy (black arrow) of renal tubules with parenchymatous degeneration of cells of renal tubules (H.&E., X400)

DISCUSSION

The cashew nut oil in this study is low in oxalate, tannins, trypsin, phytate and alkaloids all of which within the recommended WHO standard for edible food. The low values of anti-nutrients give an indication of the suitability of cashew nut oil for consumption. Also, the mean values of packed cell volume (PCV), haemoglobin content, total protein, total white blood cell (WBC), total red blood cell (RBC), neutrophil, lymphocytes, monocytes and Eosinophil of rats in this study are within the acceptable reference ranges for rodents.

The value for the lymphocyte was higher in the test group in comparison to the control, while the eosinophil value fell within normal range even with increasing dosage, indicating that the constituents that make up the cashew nut oil did not suppress the immune system of the rats as the dosage increase. Teguia *et al*. (2007) reported fluctuation in lymphocyte count suggesting that the extract might not have exerted challenges on the immune system of the animals. In a similar view, elevated lymphocyte counts in rats treated with *Garcinia kola* extract suggests that the plant may contain some constituents that enhance immune function (Aprioku, 2018). A slight drop in WBC count of rats in this study is not fully understood, but it is suggestive that the oil is not toxic to the rats. The RBC values increased slightly as dosage increase in this study. This was also the case with Haemoglobin content, suggesting that there was no adverse change in the oxygen-carrying capacity of the blood and the amount of oxygen delivered to the tissue following cashew nut oil ingestion. It is also likely that cashew nut oil does not induce anaemia at the concentration administered to the rats since these variables (RBC count and haemoglobin content) are anaemia markers. The eosinophil, lymphocyte and monocyte values of rats in this study were slightly higher in test group compared to the control group, suggesting that the rats possess high immunity against possible negative effect arising from consumption of cashew nut oil. In contrast, Chebaibi *et al*. (2019) reported a significant decrease of leukocytes, eosinophils, basophils, lymphocytes and

monocytes which is a threat to the immune system. Loss of appetite *vis-à-vis* weight loss as observed among the experimental rats in group III could be associated with metabolic disturbances associated with digestion and absorption of carbohydrates, proteins and lipids. Klaassen (2001) reported that loss of appetite is often synonymous with weight loss due to disturbances in carbohydrate, protein and fat metabolisms.

The vacoularization of hepatocytes was observed in the histology of liver tissue of wistar rats in both the control and test groups. This is a pointer to likely imbalance between the rate of synthesis of substances in the parenchymal cells and the rate of their release into the systemic circulation. Increased vacuolization or necrosis observed particularly in rats fed 8.13mL/kg of cashew nut oil in this study could be a sign of degenerative process thereby suggesting metabolic damage as a result of the amount of oil administered. The occurrence of fatty changes suggested inhibition of some lipid metabolic enzymes, thereby causing a disturbance in metabolic activity required for maintenance of tissue. Prominent fatty changes with necrosis in portal areas indicate that some toxic metabolites may be transported from the intestine to the liver, resulting in these changes. Benjamin *et al*. (2006) reported that the presence of definite necrosis indicates the presence and capability of toxic metabolites which may cause death of cells in liver, kidney and muscles of rats.

This present study also reveals that the kidney of wistar rats fed cashew nut oil exhibit signs of histopathological deformations which varied in intensity based on the amount of oil administered. These signs include shrinkage of glomeruli, glomerular distortion, necrotic renal tubules, severe congestion and atrophy of renal tubules. The highest amount administered showed more obvious toxicity in comparison to the other treatments. Therefore, as the concentration of the cashew nut oil increased, the damages done to the kidneys of the rats were more obvious.

CONCLUSION

The haematopoietic systems of the rats were not suppressed or compromised as haematological parameters fell within the acceptable reference ranges. Lymphocyte count, an immunological indicator, was slightly higher in the test group (58.17%) compared to the control group (54%) with significant increase in RBC counts and haemoglobin concentration with dose increase. However, formation of microvesicular steatosis and sinusoidal dilation in the liver tissue as well as increased pigmentation and distortion of renal tubules of the kidneys observed in this study indicates that consumption of ≥8.13 mL/kg of the oil daily over a long period of time may be deleterious and may pose serious threat to health.

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