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Original Research Article

Aju-Mbaise Decoction Improves Haematological and Kidney Markers in High-Fat Diet-Fed Wistar Rats

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ABSTRACT

Obesity has become an epidemic and prevalent disease in the world, contributing to other deadly complications worldwide with a high morbidity and mortality rate. The use of "Aju-Mbaise" decoction (AMD) in weight reduction is very common in southeastern Nigeria. This study was aimed at evaluating the possible effects of "Aju-Mbaise" decoction (AMD) on haematological and kidney markers alterations in high-fat-fed Wistar rats. Thirty male Wistar rats weighing 120-150 g were placed in 6 groups of 5 rats as follows: normal control, high-fat-fed (HFF) control, HFF rats received 5 mg/kg body weight (bw)of orlistat, and HFF rats treated orally with 5, 10, and 20 mL/kg bw of the AMD. The kidney and haematological parameters were analyzed following standard procedures. Our result revealed significant decreases in the serum urea, creatinine, and uric acid levels of AMD treated groups. The AMD showed a significant increase in red blood cell count of the treated groups. Sodium electrolyte showed a dose-dependent decrease in AMD treated groups compared to the untreated group. Red blood cell (RBC) and platelet counts were significantly elevated in AMD treated groups compared to the untreated. Treatment with AMD gave a non-significant ($p > 0.05$) elevation in lymphocytes and Eosinophil counts of the treated groups compared to the untreated control group. The administration of AMD did not affect the kidney histology. This study demonstrates that the administration of AMD had no deleterious effects on the haematology, and kidney markers as revealed by kidney histology and could be used in the treatment of diseases.

Keywords: Aju-Mbaise, Electrolytes, Haematology, Histology, Kidney markers, Red blood cell.

Introduction

Obesity is majorly caused by excessive caloric intake (dietary intake) relative to energy expenditure. Other etiology of obesity includes genetic, physiology, environmental, psychological, social, and economic factors interacting in varying degrees to promote the development of obesity.[1] The quest for nutrition has shifted from a healthy and balanced diet to highly affordable and accessible caloric and fat-laden foods that are ubiquitous at fast-food restaurants. These high-calorie food products have increased daily caloric intake with a concomitant increase in obesity. Obesity is a major risk factor associated with the development of metabolic syndromes and respiratory disorders; and could cause life-threatening diseases which may progress to cancerous stage.[2-4] Obesity can be managed with behavioral modification, pharmacologic therapy, and bariatric surgery.[5]

Stemming from the drawbacks associated with these orthodox medicines such as their effects and possible abuse and dependency, there necessitates a search for natural products from plants. There has been a tremendous increase in plant research because of its medicinal potentials attributed to the presence of bioactive compounds in them.

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The different plants that constitute AMD have been identified and comprise of leaves, roots, and trunk of medicinal plants wrapped together and used to detoxify the womb after postpartum.[6] Traditional claims have it that it has antimicrobial activity against gram-negative bacteria.[7] It has been reported to be rich in phytoconstituents which exhibited antibacterial activities against different bacteria strains and as well could be toxic to the liver on chronic administration on rats.[8] Other uses of Aju Mbaise include: uterotonic and tocolytic agents,[9] hematopoietic potencies,[10] and womb cleansing during the postpartum period.[11]

Notwithstanding the traditional claims surrounding the use of AMD in weight reduction management, there is a need to evaluate the blood-boosting potentials of AMD and possibly deleterious effects on the renal markers, hence this study.

Materials and Methods

Collection and preparation of plant materials

The polyherbal plant locally known as "Aju-Mbaise" was purchased from Eke-Amuzi Market in Amuzi-Ahiara, Mbaise Local Government Area of Imo State, Nigeria. The polyherb Aju-Mbaise was washed with salt water to remove contaminants, rinsed with distilled water, and air-dried afterward. The dried poly herb was ground into powder using an electric blender. The ground polyherb powder (50 g) was weighed into a conical flask; 250 mL of water boil at 60°C was measured into the flask containing the sample. The mixture was stirred gently for several minutes to obtain a homogenous mixture and filtered. The filtrate (decoction) was stored in a refrigerator. This

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process was repeated every two days to obtain a fresh decoction sample for administration to the rats.

High-fat diet formulation
The high-fat-diet formulation was carried out using the modified method described by Picchi *et al.*[12] The composition of the diet include carbohydrate (white corn)-200 g/kg, protein (soybean- 100 g/kg and crayfish 50 g/kg), fat (beef tallow)-550 g/kg, fibre-50 g/kg and vitamin/mineral mix-50 g/kg (Bioadd). Chukun finisher (Enugu, Nigeria); a commercial rat chow served as the standard feed.

Experimental animals
Thirty (30) male albino rats within the range of 120-150 g purchased from the rat farm in the Faculty of Veterinary Medicine University of Nigeria, Nsukka were used for the study. The animals were acclimatized for 14 days with free access to water and food before the start of the experiment. The animals were then randomly separated into six (6) groups of five (5) animals each and housed in separate aluminum cages (compartmentalized); provided with standard "pelletized" feed (Chukun "finisher" Enugu, Nigeria) and water *ad libitum* at 25^{o}C.

Ethical approval
The laboratory was maintained in good working conditions and the animals handled under the national and international ethical recommendations for care and use of laboratory animals.[14] Ethical clearance (UNN/FBS/EC/1034) was granted by the Faculty of Biological Sciences Ethical and Biosafety Committee.

Experimental design
The albino male Wistar rats were individually caged and subjected to the same dietary regimen during the two weeks of acclimatization, after which they were randomly classified and placed in 6 groups of 5 rats as follows: normal control, high-fat-fed (HFF) control, HFF rats treated with 5 mg/kg body weight of orlistat, and HFF rats treated orally with 5, 10 and 20 mL/kg body weight of the AMD. The blood-boosting potentials of AMD and possible toxicity effects of AMD on the kidney markers as well as the histology of the kidney were analyzed at the end of the study.

Induction of obesity using HFD
The induction of obesity in the rats followed the method described by Wang *et al.*[13] using the high-fat diet with slight modifications. The induction of HFD was six weeks while treatment with AMD lasted for 14 days. On completion of the study, the animals fasted overnight after which they were anesthetized with chloroform. Blood samples were collected from the retro-orbital sinus and placed in EDTA and non-heparinized tubes while the kidney was harvested for histological examination.

Haematological analysis
The whole blood in the EDTA tubes was centrifuged for 10 min at 3000 rpm. The supernatant devoid of particles was used for haematological analysis.[15]

Kidney markers analysis
Total protein, creatinine, urea, and uric acid concentrations were determined using test kits (Randox Laboratories Ltd., Crumlin, United Kingdom) as described by Mayne.[16] The plasma sodium, potassium, chloride, and calcium ion concentrations were determined using test kits (TECO diagnostics, USA) as described by Gross *et al.*[17]

Histological analysis
The histological examination of the experimental animal prostate tissues was carried out following the procedure described by Apeh *et al.*[18] The tissues were fixed in 10% phosphate-buffered formalin for about 48 hours. Subsequently, they were prepared for histopathological examination using standard techniques. The slides were examined with a Motic® light microscope and the photomicrographs were taken using the Motic® microscope camera.

Statistical analysis
The results are expressed as mean ± standard deviation for five rats per group (n = 5). The data obtained were analyzed using the Statistical Product and Solution Service (SPSS) version 23. One-way analysis of variance (ANOVA) was used for comparison across the different groups and differences were significant at $p < 0.05$. *Post-hoc* was used for separating and comparing the means.

Results and Discussion

Effect of AMD on haematological indices of HFF Wistar rats
Treatment of the HFF rats with AMD significantly elevated the platelets and red blood cell (RBC) counts in the high dose group compared to the untreated control. White blood cell (WBC), packed cell volume (PCV), and lymphocyte counts were not affected by the administration of AMD. Also, the neutrophil and eosinophil counts were not affected by the administration of AMD when compared to the normal control (Table 1).
This increased red blood count in our study is consistent with the report of Ohlsson and Aher,[19] According to Isaac *et al.*[20] RBC transports oxygen and carbon dioxide in the body. Therefore increase in the level of RBC count in the groups treated with AMD (Table 1) implies an increase in the level of oxygen circulated to the tissues and likewise of carbon dioxide moved to the lungs.[21,22] This leads to a decreased risk of obesity hypoventilation syndrome, a breathing disorder in obese people. White blood cells play a critical role in boosting the immune system functionality.[20] This suggests that the significant decrease observed between the untreated control and the groups treated with AMD caused the reduction in the white blood cell response to inflammatory conditions as postulated by Chmielewski and Strzelec,[23] Thus, animals with high WBC comprising of neutrophil, lymphocytes, and eosinophils indicates a disease state manifestation and are exposed to high risk of disease infection, while those with low counts form a strong defense mechanism that is resistant to diseases. This observation suggests that AMD administration had no toxic effect. The non-significant difference observed in PCV followed the same trend in the work of Evbakhavbokun *et al.*[8] and Nnadiukwu *et al.*[10] who in their independent research reported that Aju Mbaise administration did not affect the PCV of rats. Also, a significant rise in platelet counts followed the same trend in our study. This suggests that AMD detoxifies and aggregates foreign components and stabilizes the blood towards the transport of oxygen in the body system.

Effect of AMD on some kidney parameters in HFF Wistar rats
The result of AMD on the kidney functions was presented in Table 2. There was a significant decline in the serum urea, and uric acid levels of the treated groups compared to the normal and untreated groups. Serum creatinine level was not affected by the administration of AMD when compared to the controls. Total protein was elevated in the mid dose treated group compared to the untreated group.
The significant reduction in the urea concentration did not follow the reports of Ray *et al.*[24] who documented elevation in serum urea level of obese individuals. One of the risks of obesity is kidney damage,[25] normally; urea is eliminated from the blood by the kidney into the urine, making the serum urea level to be low in the blood and high in the urine. When the kidney is damaged, the ability of the kidney to excrete urea diminishes. The reduction of urea in the groups treated with AMD showed the effectiveness of the polyherb in reducing kidney damage. The reduction in the serum uric acid could be likened to an increased fat mass in the untreated control. When individuals are obese it exposes them to high-altitude-induced hypobaric hypoxia.[26] Hypoxic conditions (low oxygen saturation) lead to hyperuricemia (an increase in uric acid levels).[27] Therefore, it can be said that the hypoxic condition in obese individuals predisposes them to hyperuricemia.[28] The increase in total protein levels could be attributed to the presence of a large amount of fat mass in untreated rats. This is because an additional increase in protein intake could be effective in maintaining a fat-free body mass. An increase in the fat mass of a rat may lead to a decrease in total protein.[29]

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Effect of AMD on serum electrolyte concentrations in HFF Wistar rats
The result of AMD on the serum electrolytes was presented in Table 3. The administration of AMD significantly lowered serum sodium ion concentrations in all the treated groups compared to the untreated control. There was no significant difference in potassium, chloride, and calcium ion concentrations compared to normal and untreated control.
The reduction of sodium level by Aju Mbaise decoction showed an indication of a low risk of hypertension, as obesity is one of the risk factors of hypertension.[30] Serum chloride ion was not significantly lowered in all the treated groups compared to the control groups. In this study, the elevated serum sodium ion was balanced by the concomitant decrease in the chloride ion in body fluids that carries electrical impulses and are vital in keeping the functionality of the cardiac muscles.[31]

Effects of AMD on the kidney histology of the HFF Wistar rats
The histology of the kidney of rats treated with AMD was represented in Figure 1. The cross-sections of the kidney treated with AMD revealed the normal renal corpuscles and distal convoluted tubules.
The result of Aju-Mbaise on the kidney shows that the different doses of Aju-Mbaise and the standard drug used has no negative effect on the kidney. This disagrees with the report of Onyejike, *et al.*[32] From their findings, Goko cleanser herbal mixture was toxic to the kidney and causes a decrease in body weight and food consumption.

Table 1: Haematological parameters of rats treated with AMD

Groups/parameters	Platelets (10^9/L)	RBC (10^{12}/L)	WBC (10^9/L)	PCV (%)	Lymphocytes (10^9/L)	Neutrophil (10^9/L)	Eosinophil (10^9/L)
Normal control	$3.17 \pm 0.15^{b,c}$	3.87 ± 0.58^{a}	5.33 ± 0.31^{a}	42.67 ± 0.58^{b}	40.67 ± 9.24^{a}	58.67 ± 9.87^{a}	0.67 ± 1.15^{a}
Untreated control	$2.85 \pm 0.28^{a,b,c}$	3.87 ± 0.15^{a}	6.93 ± 0.31^{b}	42.67 ± 0.58^{b}	32.00 ± 5.29^{a}	67.33 ± 4.62^{a}	0.67 ± 1.15^{a}
Standard control	2.67 ± 0.03^{a}	3.90 ± 0.10^{a}	6.33 ± 0.51^{b}	43.33 ± 0.58^{b}	38.67 ± 8.08^{a}	60.00 ± 9.17^{a}	1.33 ± 1.15^{a}
Low dose	3.21 ± 0.03^{c}	4.43 ± 0.21^{c}	4.73 ± 0.50^{a}	36.67 ± 1.15^{a}	37.33 ± 6.43^{a}	61.33 ± 7.57^{a}	1.33 ± 1.15^{a}
Mid dose	$2.78 \pm 0.04^{a,b}$	4.17 ± 0.15^{b}	5.13 ± 0.31^{a}	37.00 ± 1.00^{a}	44.00 ± 12.17^{a}	54.00 ± 14.00^{a}	1.33 ± 1.15^{a}
High dose	$3.21 \pm 0.38^{b,c}$	$4.20 \pm 0.10^{b,c}$	6.33 ± 0.50^{b}	43.00 ± 1.00^{b}	36.67 ± 8.33^{a}	62.67 ± 8.08^{a}	0.67 ± 1.15^{a}

Values are expressed as mean ± SD, (n = 5). Values in the same column having different superscripts letters differ significantly ($p < 0.05$).
Normal Control: Not induced, not treated; Untreated Control: high-fat-fed (HFF) control; Standard Control: HFF rats treated with 5 mg/kg body weight of orlistat; Low Dose: HFF rats treated orally with 5 ml/kg AMD; Mid Dose: HFF rats treated orally with 10 ml/kg AMD; High Dose: HFF rats treated orally 20 ml/kg body weight of the AMD.

Table 2: Effect of AMD on some kidney parameters in HFF Wister albino rats
Values are expressed as mean ± SD, (n = 5). Values in the same column having different superscripts letters differ significantly ($p < 0.05$).

Groups	Urea (mmol/L)	Creatinine (µmol/L)	Total Protein (g/dL)	Uric acid (µmol/L)
Normal control	$40.05 \pm 8.64^{a,b}$	1.25 ± 0.05^{a}	4.53 ± 0.19^{b}	5.96 ± 1.97^{b}
Untreated control	49.74 ± 6.26^{b}	1.28 ± 0.02^{a}	3.07 ± 0.13^{a}	11.61 ± 1.15^{c}
Standard control	$38.31 \pm 5.11^{a,b}$	1.24 ± 0.06^{a}	3.67 ± 0.38^{a}	3.87 ± 0.82^{a}
Low dose	$38.99 \pm 5.66^{a,b}$	1.23 ± 0.06^{a}	3.39 ± 0.53^{a}	7.53 ± 0.43^{b}
Mid dose	36.45 ± 4.69^{a}	1.27 ± 0.16^{a}	4.63 ± 0.59^{b}	$5.65 \pm 1.14^{a,b}$
High dose	36.62 ± 7.48^{a}	1.24 ± 0.04^{a}	3.52 ± 0.36^{a}	6.71 ± 0.09^{b}

Normal Control: Not induced, not treated; Untreated Control: high-fat-fed (HFF) control; Standard Control: HFF rats treated with 5 mg/kg body weight of orlistat; Low Dose: HFF rats treated orally with 5 ml/kg AMD; Mid Dose: HFF rats treated orally with 10 ml/kg AMD; High Dose: HFF rats treated orally 20 ml/kg body weight of the AMD.

Table 3: Effect of AMD on serum electrolyte concentrations in HFF Wistar rat

Groups	Na^+ (mEq/L)	K^+ (mEq/L)	Cl^- (mEq/L)	Ca^{2+} (mEq/L)
Normal control	257.76 ± 7.67^{a}	3.97 ± 0.04^{bc}	109.00 ± 0.10^{b}	10.93 ± 0.77^{ab}
Untreated control	311.25 ± 2.99^{c}	3.15 ± 0.26^{a}	109.00 ± 3.61^{b}	10.64 ± 0.25^{b}
Standard control	266.72 ± 13.77^{ab}	4.15 ± 0.34^{c}	106.00 ± 4.36^{ab}	$12..35 \pm 1.30^{b}$
Low dose	295.72 ± 18.84^{b}	3.69 ± 0.32^{bc}	101.33 ± 1.15^{a}	10.77 ± 0.45^{a}
Mid dose	$259.20 \pm 29.46^{a,b}$	3.53 ± 0.25^{ab}	103.00 ± 5.20^{ab}	11.03 ± 0.07^{ab}
High dose	278.12 ± 25.96^{abc}	3.54 ± 0.23^{ab}	108.00 ± 0.10^{b}	11.12 ± 0.93^{ab}

Values are expressed as mean ± SD, (n = 5). Values in the same column having different superscripts letters differ significantly ($p < 0.05$).
Normal Control: Not induced, not treated; Untreated Control: high-fat-fed (HFF) control; Standard Control: HFF rats treated with 5 mg/kg body weight of orlistat; Low Dose: HFF rats treated orally with 5 ml/kg AMD; Mid Dose: HFF rats treated orally with 10 ml/kg AMD; High Dose: HFF rats treated orally 20 ml/kg body weight of the AMD.

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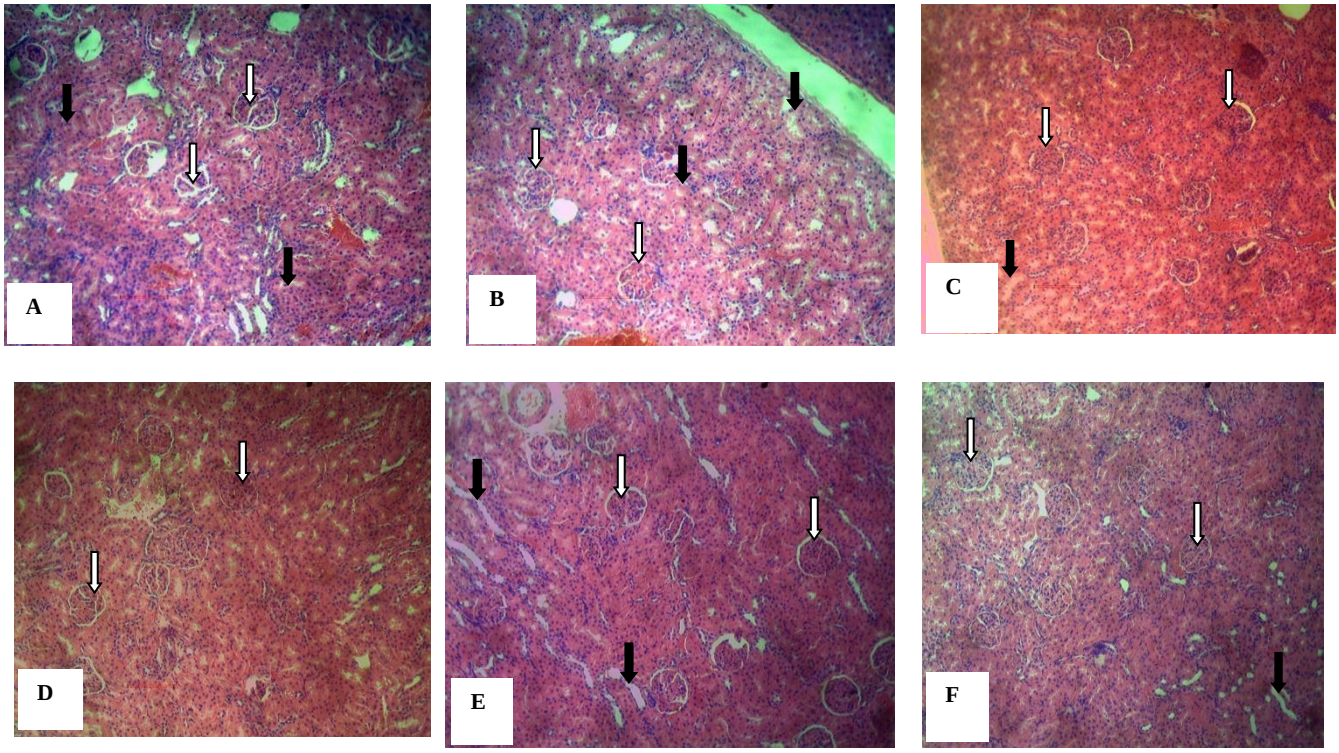

Figure 1: Cross-section of the kidney of HFF Wistar rats treated with AMD. The cross-section of the kidney (A-F) shows normal renal corpuscles (white arrow), and myriads of normal renal tubules, proximal and distal convoluted tubules (black arrow). (H & E 100×)

Conclusion

Our study demonstrates that the AMD had no toxic effect on the kidney as revealed by the kidney histology. It boosted the haematological parameters which are in the front line of fighting invading pathogens in the immune system. The identification of the active principles in AMD is vital and may be subjected to clinical trials which might be a new dawn in the development of effective anti-obesity agents that are not toxic to the body system.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

References

1. Aronne LJ, Nelinson DS, Lillo JL. Obesity as disease state: a new paradigm for diagnosis and treatment. Clin Cornerstone. 2009; 9(4):9-29.
2. Fleming T and Robinson M. "Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: A systematic analysis for the Global Burden of Disease Study 2013," Lancet**.** 2014; 384(9945):766–781.
3. Landsberg L, Aronne LJ, Beilin LJ. "Obesity-related hypertension: Pathogenesis, cardiovascular risk, and treatment—a position paper of the obesity society and the American society of hypertension," Obesity, 2013; 21(1):8–24.
4. Obeta A, Okauchi S, Kimura T. "Advanced breast cancer in a relatively young man with severe obesity and type 2 diabetes mellitus," J Diabetes Invest. 2017; 8(3):395-396.
5. Temple VJ. Biochemistry 1001: Review and Viva Voce Questions and Answers Approach, Sterling Publishers Private Limited, New Delhi. 2012. 30 p.
6. Nnadiukwu AT, Monago-Ighorodje CC, Chukwu LC. Phytochemical Composition of Ethanol Extract of a Cocktail Herbal Mixture (Aju Mbaise). J Adv Biol Biotechnol. 2019; 22(1):1-11.
7. Ogueke CC, Owuamanam CL, Onyedinma C, Iroanya A, Bede EN, Nwachukwu IN. Antibacterial activity, Phytochemical properties and mineral content of "Aju Mbaise" decoction: A liquid extract administered to nursing mothers. Niger J Nutr Sci. 2016; 37(1):114-121.
8. Evbakhavbokun O, Ekrakene T, Daniel EO, Olisaka FN. Phytochemical Screening, Liver Toxicity and Antibacterial Properties of Aqueous and Ethanol Extracts of a Local Herbal Product "Aju Mbaise". Trop J Nat Prod Res. 2020; 4(6):237-242.
9. Ijioma SN, Osim EE, Nwankwo AA, Kanu KC, Orieke D. Southeast Nigerian polyherbal (AJUMBISE): A potential uterotonic and tocolytic agents. Sci Afr. 2020; 8:e00393.
10. Nnadiukwu AT, Monago-Ighorodje CC, Chukwu LC. Haemoglobin and packed cell volume (PCV) of high-fat diet/steptozotocine-induced diabetic Wistar rats treated with ethanol extract of a herbal mixture (Aju Mbaise). Int Blood Res Rev. 2019;9(4):1-6.
11. Iwueke VA and Chukwu CE. Ethnotherapeutic Profile of Medicinal Plants Used during Postnatal Recovery (Postpartum) in South Eastern Nigeria: A Review. Arch Curr. Res. Int. 2020; 20(5):31-41.

12. Picchi MG, De Mattos AM, Barbosa MR, Duarte CP, Gandini MA, Portari GV, Jordao AA. A high fat diet as a model of fatty liver diseases in rats. Acta *Cirurgica Brasileira*. 2011; 26(2):1678-2674.
13. Wang T, Wu Q, Zhao T. Preventive Effects of Kaempferol on High-Fat Diet-Induced Obesity Complications in C57BL/6 Mice. Biomed Res Int. 2020; Article ID 4532482.
14. National Academy of Sciences [NAS]. Guide for the care and use of laboratory animals. 8th ed. Washington DC: National Academy of Sciences. 2011.
15. Ochei J and Kolhatkar A. Medical Laboratory Sciences; Theory and Practice. Tata McGraw-Hill Publishing Co. Ltd. New Delhi; 2008. 321-324 p.
16. Mayne PD. Clinical Chemistry in Diagnosis and Treatment. 6th Edn., Arnold London/Oxford University Press Inc., New York. 1996; 205-248 p.
17. Gross P, Benzing T, Hensen J, Monig H. Practical approach to hyponatremia. Deut Med Wochenschr. 2011; 136(34-35): 1728–1732.
18. Apeh VO, Amoke CO, Anaduaka EG, Ohayi SAM, Egba SI, Agu CV, Uzoegwu PN. Biochemical and histopathological studies on the effects of raw and cooked *Tetracarpidium conophorum* on male albino rats. Br J Med Med Res. 2015; 5(8):1024-1033.
19. Ohlsson A and Aher SM. Early erythropoietin for preventing red blood cell transfusion in preterm and/or low birth weight infants. Cochrane Database Syst Rev. 2012; 9:4863.
20. Isaac LJ, Abah G, Akpan B, Ekaette IU. 2013. Haematological properties of different breeds and sexes of rabbits. Proceedings of the 18th Annual Conference of Animal Science Association of Nigeria, 2013; 24-27 p.
21. Ugwuene MC. Effect of Dietary Palm Kernel Meal for Maize on the Haematological and Serum Chemistry of Broiler Turkey. Niger J Anim Sci. 2011; 13:93-103.
22. Soetan KO, Akinrinde AS, Ajibade TO. 2013. Preliminary studies on the haematological parameters of cockerels fed raw and processed guinea corn (*Sorghum bicolor*). Proceedings of 38th Annual Conference of Nigerian Society for Animal Production. 2013. 49-52 p.
23. Chmielewski PP and Strzelec B. Elevated leukocyte count as a harbinger of systemic inflammation, disease progression, and poor prognosis: a review. Folia Morphol. 2017; 77(2):171–178.
24. Ray AS, Kare PK, Makwane HS, Saxena T, Garg C. Estimation of serum creatinine, serum urea, glomerular filtration rate and proteinuria among apparently healthy adults to assess the renal impairment and its association with body mass index: An observational hospital-based study. Int J Med Res Rev. 2020; 8(2):183-188.
25. Edmund L and David J. Kidney function tests. Carl A.B., Edward R, and David E. (Eds.) Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. 4th ed. New Delhi. Elsevier Inc. 2006. 797-808 p.
26. Verdich C, Toubro S, Buemann B, Holst JJ, Bulow J. Leptin levels are associated with fat oxidation and dietary-induced weight loss in obesity. Obes Res**.** 2001; 9:452-461.
27. Woolliscroft J, Harry C, Fox IH. Hyperuricemia in acute illness: a Poor prognostic sign. Am J Med. 1982; 72:58-62.
28. Ri-Li G, Chase PJ, Witkowski S, Wyrick BL, Stone JA, Levine BD, Babb TG. Obesity: associations with acute mountain sickness. Ann Intern Med. 2003; 139(4):253-270.
29. Mathijs D, Lea T, Blandine GC, Tanja A, Margaret WP. Dietary protein and energy balance in relation to obesity and co-morbidities. Front Endocrinol. 2018; 9:443.
30. Ahmad IM, Wudil AM, Yunusa I. Effect of Oral Administration of Aqueous Leaves Extract of *Vitex doniana* on Serum Electrolyte Levels in Rats. Pak J Biol Sci. 2013; 16:1819-1822.
31. Seldin DW and Rector RC. The generation and maintenance of metabolic alkalosis. Kidney Int. 1972; 1: 306–321.
32. Onyejike DN, Aladeyelu DN, Onyejike SO, Miracle I. Histopathological of Goko cleanser on the kidney. Int J Innov Res**.** 2018; 5:255-345.