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Fresh Juice of *Telfairia occidentalis* Modulates Some Biochemical and Hematological Markers in Wistar Rats Exposed to Lead Acetate

[1*]Adewale Omowumi Oyeronke, [1]Adetuyi Oluwatosin Adefunke and
[2]Samuel Ekundayo Stephen

[1] Department of Biochemistry, Faculty of Basic and Applied Sciences, Osun State University, Osogbo, Nigeria.

[2] Cancer Research and Molecular Biology Laboratories, Department of Biochemistry, College of Medicine, University of Ibadan, Ibadan, Nigeria.

* Corresponding Author: adewaleomowumi13@gmail.com

ABSTRACT

The modulatory effect of fresh juice of *Telfairia occidentalis* (FJTO) on biomarkers of liver and kidney functions was investigated in rats exposed to lead acetate. Male wistar rats were exposed to either distilled water, 100 mg/kg bwt of FJTO, 60 mg/kg bwt of lead acetate or combination of FJTO and lead acetate orally and daily for 14 days. Experimental assays revealed that lead acetate caused significant increase ($p<0.05$) in serum transaminases and alkaline phosphatase activities, concentrations of blood urea nitrogen (BUN), creatinine, potassium and sodium. Significant decrease ($p<0.05$) in serum total protein and glucose concentration with histological changes in the liver and kidney were also observed. Co-administration with FJTO significantly modulated these observations. The study revealed the ability of FJTO to preserve the crucial function of liver and kidney of rats exposed to lead acetate.

Key words: Lead acetate, biomarkers, toxicity, *telfairia occidentalis*, modulation

INTRODUCTION

Environmental pollution is a major concern to public health, as several toxic industrial raw materials, products and their metabolites continuously escape into the environment, ranging from the atmosphere, the aquatic system and soil [1]. The exposure of human and animal population throughout the world on daily basis to low levels of environmental contaminants has been reported [2] which establishes the fact that direct or indirect contact with these substances are unavoidable.

Some of these substances are inevitably used in different industries, laboratories, automobile companies or for agricultural purposes as a result of their crucial properties that boost activities in these sectors [3].

Lead is one of the very important materials used abundantly in several industries globally. It possesses important qualities such as being able to be molded into different shapes, poor conductibility which prevents electrocution hazards, ability to form thin rods, and resistance to corrosion [4]. These properties increase the usefulness of lead. However, lead as a metal alone or with some other atoms have been reported to be highly hazardous [5]. Because lead is not affected by bacterial degrading ability, its pure form and its compounds tend to accumulate in the environment thereby resulting in a lot of environmental hazards. Though, a discontinuous widespread use of lead in many sectors has been advised, human exposure to lead and its compounds is yet far from limitation due to the aforementioned qualities [6].

In the course of induction of toxicity by lead and related compounds, the organs most importantly targeted have been the kidney and the liver owing to their critical roles in detoxification and metabolism of endogenous substances [7]. Since, it has been established that continuous exposure to at least low levels of lead and its related compounds is not negotiable, continuous search for antidotes that will preserve these susceptible organs from the toxicities caused by lead will not be considered a misplaced research priority.

However, from distant years, plants, especially vegetables, have been considered a rich source of nutritional and therapeutic agents combating several ailments including toxicant related diseases [8]. *Telfairia occidentalis* popularly known as fluted gourd, fluted pumpkin, and Ugu by many [9] is one of the vegetables commonly consumed for its nutritional and medicinal purposes especially among Nigerians. Series of studies have confirmed among others, the anti-oxidative, neuro-protective, hepato-protective and nephro-protective activities of different extract of *Telfairia occidentalis* [10-12]. *T. occidentalis* is reported for its high superoxide and hydroxyl radical scavenging potentials which could be part of justification for its medicinal qualities. Furthermore, the protective effect of its extract against drug-induced organ damage have been established [12].

Fresh juice of *Telfairia occidentalis* has been known to be blood booster and to possess several medicinal qualities including anti-inflammatory. However, it is not known whether fresh juice of

Telfairia occidentalis can alter the toxic effect induced by lead or its compounds. Therefore, this study was designed to assess the modulative effect of fresh juice of *Telfairia occidentalis* on lead acetate-induced liver and kidney toxicities.

MATERIALS AND METHODS

Plant materials

Fresh young leaves with stalks of *Telfairia occidentalis* were obtained from the vegetable market in Oke Baale, Osogbo, Nigeria. The plant was identified and authenticated with voucher number UIH-22847 at the Herbarium of Department of Botany, Faculty of Science, University of Ibadan, Nigeria.

Lead acetate (CH_3COO) $2Pb.3H_2O$, mol.wt 379.33, 99.999%, CAS No. 6080-56-4 was manufactured by Aldrich Chemical Co. Inc. St. Paul Avenue Wisconsin USA). All other reagents and chemicals used were of analytical grade and were obtained from Sigma Chemical Co. St. Louis, Mo. USA.

Animals

Twenty (20) adult male albino rats were procured from the animal house of the Department of Biochemistry, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria. They were kept in cages, at room temperature (24 -27^{o}C), relative humidity 60 -70%, and 12-hour light/dark cycle. Commercial rat feeds and water were available *ad libitum*. The guidelines on humane handling of animal was approved by the Ethics Committee of the College of Health Sciences, Osun State University Osogbo

Plant extract preparation

The fresh *Telfairia occidentalis* leaves with stalks were washed with clean water and drained dried. They were then homogenized to paste using cleaned mortar and pestle and a calculated volume of distilled water was added. The resulting paste was then placed on a clean muslin cloth to collect the aqueous extract which was measured. The extract was administered at the dose of 100 mg/kg body weight. The extract was prepared fresh throughout the period of the experiment.

Experimental design

The animals were acclimatized for two weeks after which they were randomly divided into four groups of five animals each. Control rats were given 0.5 ml/kg body weight distilled water; rats

in Pb group were given 60 mg/kg body weight of lead acetate; rats in FJTO group were given 100 mg/kg body weight of fresh juice of *Telfairia occidentalis* and rats in FJTO + Pb group were given 100 mg/kg body weight of fresh juice of *Telfairia occidentalis and* 60 mg/kg body weight of lead acetate simultaneously. The test samples were orally administered once daily for 14 days. Lead acetate was dissolved in distilled water and administered at a dose of 60 mg/kg. The dose of lead acetate dosage was based on the study of Sujatha et al [13] and it corresponds to $1/10^{th}$ of the reported LD_{50} while the dose of FJTO was formerly used by Salman et al. [14]. After the treatment period, animals were fasted overnight and sacrificed 24 hours after the last dose under ketamine and xylazine anaesthesia. Blood samples were obtained retro-orbitally, a portion was collected in heparinized bottles for haematological analysis. The other portion of blood collected in plain bottles was centrifuged at 3000g for 10 minutes to obtain clear non-hemolyzed sera which were stored at -20^{o}C for biochemical analysis. The liver and kidney tissues were quickly excised and washed in 1.15% cold KCl and blotted on filter papers to remove adhering blood, weighed and fixed in 10% formalin for histological examination.

Determination of hematological parameters

Hemoglobin (Hb) concentration was determined according to Cyanmethemoglobin method [15]. White Blood Cell Counts (WBC), Packed Cell Volume (PCV), Red blood cell (RBC) count, Lymphocytes % (L%), platelets and Neutrophils % (N%) were determined by following Hemocytometer procedure.

Determination of serum hepatic function biomarkers

The hepatic function biomarkers: ALT and AST, were determined according to the principle reported by Reitman and Frankel [16]. GGT and ALP were determined following the principles reported by [17] and [18] respectively. Total protein concentrations of the serum were determined according to Biuret method as described by Gornall et al [19]. Albumin concentrations were determined following the principle reported Gornall et al [19]. Triglycerides concentration was determined by following the principle reported by Bucolo and David [20] and glucose concentration through the procedure reported by Medina et al. [21].

Determination of renal function biomarkers

The renal function biomarkers: BUN and creatinine concentrations were determined following the principles described by Weatherburn [22] and Henry [23] respectively. Serum sodium and potassium concentrations were obtained following the method described by Maruna and Trinder [24] with slight modification.

Histopathological Analysis

The liver and kidney were excised from the animals after sacrificed and fixed in 10% formalin solution, for tissue sections and subsequent histopathological examination. The tissues were then embedded in paraffin. Rotary microtome was used to collect five micrometer-thick paraffin sections and tissues were thereafter stained with Haematoxylin and Eosin (H&E). The specimens were examined and photographed under light microscope.

Statistical analysis

The data were expressed as mean ± standard deviation after analysis by one-way analysis of variance (ANOVA) with the aid of Statistical Package for Social Sciences (SPSS) software, SPSS Inc., Chicago, Standard version 17.0. Differences between mean values of different groups were considered statistically significant at $P < 0.05$.

RESULTS AND DISCUSSION

Table 1: Effect of FJTO on hematological parameters of lead acetate-induced rats

	PCV (%)	HB(g/dl)	RBC($\times 10^6$)/uL	LYM(%)
CONTROL	37.60±3.21	12.74±1.09	6.07±0.66	71.00±3.16
Pb	37.00±4.42^{ab}	12.70±1.14^{ab}	5.87±0.92^{ab}	66.60±2.70
FJTO	50.40±3.05 ^{ab}	16.74±0.49 ^{ab}	8.41±0.46 ^{ab}	68.20±4.66
FJTO+Pb	49.20±1.10^{b}	16.68±0.61^{ab}	8.31±0.18^{ab}	68.00±3.61

The values are expressed as mean ± SD for n=5. ^{a} P<0.05 level of significant as compared to the control: ^{b} P<0.05 level of significant as compared to Pb

Table 2: Effect of FJTO and Lead Acetate on Electrolytes in rats

	BUN(m/mol)	CREATININE (mg/dl)	Na (m/mol)	K(m/mol)	ALBUMIN(mg/dl)
CONTROL	16.92±0.44	0.66±0.05	142.80±1.30[b]	5.40±0.18	3.56±0.11
Pb	19.24±0.90[b]	0.82±0.04[b]	151.4±5.94[a]	7.06±0.91[a]	2.74±0.39[a]
FJTO	16.72±0.43[a]	0.64±0.06[a]	142.80±0.83[b]	5.80±0.19[b]	3.52±0.08 [b]
FJTO+Pb	18.14±0.70[a]	0.72±0.08	144.90±1.30	5.84±0.26[b]	3.16±0.42

The values are expressed as mean ± SD for n=5. [a] P<0.05 level of significant as compared to the control: [b] P<0.05 level of significant as compared to Pb

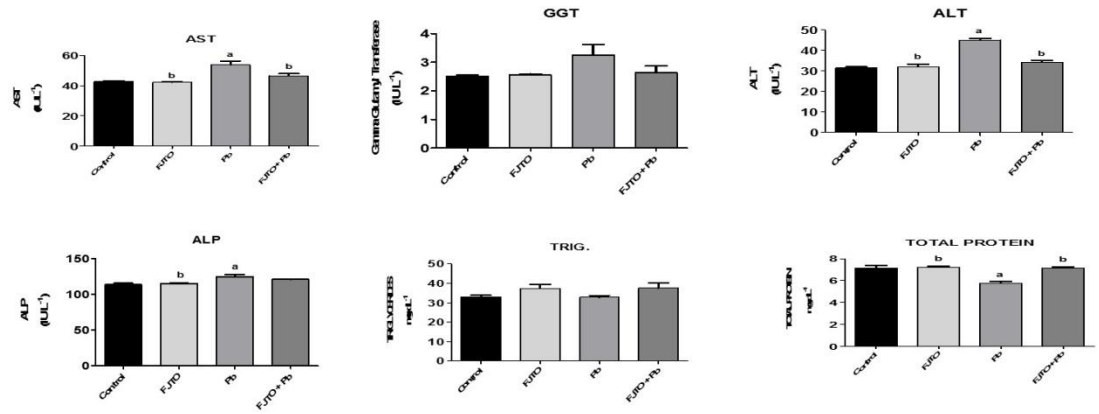

Fig 1: Effect of FJTO on liver function biomarkers in lead acetate-induced rats
The values are expressed as mean ± SD for n=5. [a] P<0.05 level of significant as compared to the control: [b] P<0.05 level of significant as compared to Pb

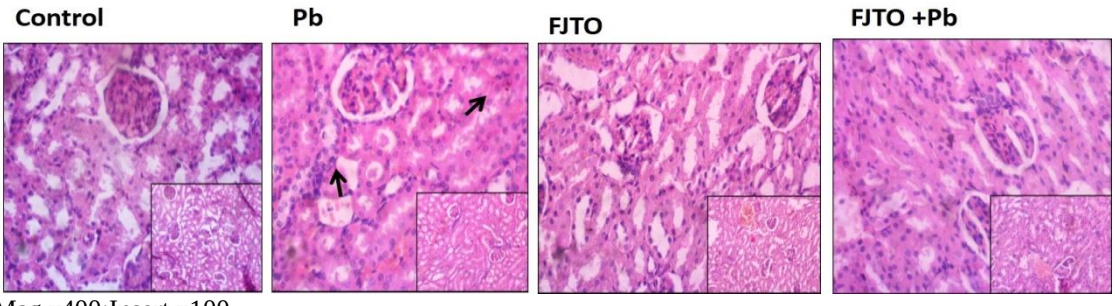

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Fig 1: Effect of FJTO on histological examination of the kidney section of lead acetate-induced rats. Control: No visible lesion; Pb: moderate congestion of tubule-interstitial spaces (arrow; FJTO.:No visible lesion FJTO + Pb; No visible lesion.

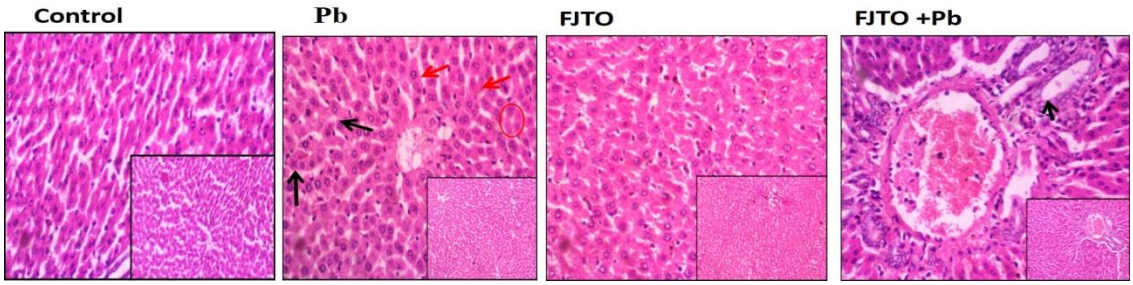

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Fig 2: Effect of FJTO on histological examination of the liver section of lead acetate-induced rats. Control: No visible lesion Pb: Vacuolar hepatocellular degeneration (black arrows) and necrosis (circled areas) around the centrilobular to mid-zonal region. Moderately increased frequency of binucleate or dividing cells (red arrows). FJTO: No visible lesion FJTO +Pb; cellular degeneration (arrow)

Human exposure to lead compounds toxicities is well documented. Therefore, continuous search for antidotes that will preserve susceptible organs from these toxicities is encouraged. The present study was undertaken to assess the modulatory role of FJTO on Pb-induced toxicity in Wistar rats. For this purpose, rats were maintained on lead (60 mg/kg BW) for 14 days and hematology, serum chemistry and histology were monitored.

Evaluation of hematological indices is pivotal in determining the lethal and deleterious impact of xenobiotics including lead and plant extracts on the blood constituents of an animal. It thus gives a correlation between such compounds and the blood relating functions they possess [25].

The exposure of rats to lead (Pb) elicited changes in blood parameters such as hemoglobin, PCV and eosinophils. However, there were no observed effects on the WBC, platelet, neutrophils, monocytes and lymphocytes. As a result, Pb exposure has little or no impact on immunity while FJTO maintains the immune system. Importantly, co-exposure of rats to Pb and FJTO, restored Hb, PCV and eosinophil levels to status comparable to the control. This is a clear indication of the selective modulation of FJTO. In this study, FJTO ameliorated the Pb-induced reduction in number of erythrocytes. This was confirmed by the increased hematocrit (PCV) and percentage Hb in the Pb + FJTO group. In normal situations, local tissue anoxia apparently leads to the formation of erythropoietin, which stimulates increased erythrocyte production [26]. This action of FJTO may be attributed to it containing erythropoietin-like agent(s) which is/are responsible for the increased production of erythrocytes. Moreover, the observed elevation of Hb and RBCs in (FJTO + Pb) group may also be due to stimulatory and compensatory effect arising from the

destruction of the blood corpuscles, which may also be implicated in the increased percentage of PCV in animals [25]. Also, the oxygen carrying capacity of the blood is maintained [28]. Our results are in tandem with Ashafa *et al*. [29]. Evaluating hepato-renal functional indices is vital to understanding toxicological effects in the liver and the kidney and Isnard *et al.* [30] and Saad *et al.* [31] have reported the impact of phytochemicals and bioactive substances on these organs. The kidney function can be assessed through indices such as electrolytes, creatinine. Also, albumin and total bilirubin are molecules used to evaluate the appropriate functioning of the liver [32]. In this study, we observed a reduction in the level of serum albumin in the Pb-exposed group. This may indicate liver malfunction, resulting from hepatocellular damage [33], thus imparing its ability for protein transport in the blood. Bilirubin is a known metabolic product of blood with biological and diagnostic values. The observed increase in total bilirubin compared with the control is an indication of impaired liver functioning [34]. However, the non-definite pattern found in the Pb + FJTO group may be explained as adaptation by the animals to the effect of the juice.

The levels of electrolytes, creatinine were also assessed in the serum of animals. The significant increased effect of Pb on the serum concentrations of sodium and potassium ions of the animals suggests an abnormal electrolyte ratio in the organ and thus an indication of impaired kidney function. It was however observed that in the Pb + FJTO group, sodium and potassium ions were modulated to safety status. Creatinine, synthesized in the liver, passes into the circulation where it is taken up almost entirely by the skeletal muscles. Its retention in the blood is an evidence of kidney impairment [35]. Therefore, the reduced levels of creatinine in the serum may imply that the extract has interfered with creatinine metabolism and its eventual excretion from the blood.

Certain enzymes such as phosphatases, dehydrogenases and transferases, found in the serum do not originate from the extracellular fluid. During tissue damage, some of these proteins find their way into the serum, probably by leakage [36], through disrupted cell membranes. Serum enzyme measurements are thus vital signs of hepatic damage. The increase in serum AST, ALT, ALP and GGT activities in the Pb-treated group implied cellular damage to the plasma membrane of the rats' organs. Such signals implicate Pb as a hepatotoxic metal. The complementary reduction in the serum enzymes may imply inhibitions at the cellular level by FJTO.

Alterations in the concentration of major lipids such as cholesterol, high-density lipoprotein cholesterol, low density lipoprotein cholesterol and triacylglycerol can provide valuable information on metabolism of lipids which takes place in the liver [37] and susceptibility to heart diseases [38]. In our study, we evaluated the Pb-induced alterations in Triacylglycerols. Triacylglycerols are the storage form of fatty acid. There were no significant effects observed in both Pb-treated and Pb+ FJTO groups. Thus, neither Pb nor FJTO predisposes to atherosclerosis and heart diseases [39] and non of the two substances affected lipid metabolism in this study.

Furthermore, results from our study show that Pb reduced blood glucose levels compared to the control, while FJTO had no effect on blood glucose levels. Our results are at variance with [40] who posited that lead aggravates blood glucose levels in male wistar rats.

Lastly, histological malformations and alterations such as vacuolar hepatocellular degeneration and necrosis around the centrilobular to mid-zonal region were induced by lead in the liver and in the kidney, moderate congestion of tubule-interstitial spaces were observed in the same group. Initially, we have reported this observation [41]**,** however, this damage was modulated in the FJTO + Pb treated group. This further confirms the ability of FJTO to modulate lead-induced alteration in both organs.

CONCLUSION

This study suggests that FJTO could exert its protective effects mainly by inhibiting biochemical and hematological alterations in rats Therefore, FJTO could be a novel therapeutic tool for the treatment of hepatic and nephro-toxicity closely associated with Pb in rats.

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REFERENCES

1. Fullerton, D. G., Bruce, N. & Gordon, S. B. (2008). Indoor air pollution from biomass fuel smoke is a major health concern in the developing world. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *102*(9), 843-851.
2. Bolognesi, C. & Morasso, G. (2000). Genotoxicity of pesticides: potential risk for consumers. *Trends in Food Science & Technology*, *11*(4-5), 182-187.

3. Bradl, H. (Ed.). (2005). *Heavy metals in the environment: origin, interaction and remediation* (Vol. 6). Elsevier.
4. Kaye & Laby Online Version 1.0 2005. In *The Technical Writer's Handbook*. University Science.
5. Mahaffey, K. R. (1990). Environmental lead toxicity: nutrition as a component of intervention. *Environmental Health Perspectives*, *89*, 75-78.
6. Gabby, P. N. (2006). Lead: in Mineral Commodity Summaries. Reston, VA. *US Geological Survey*.
7. Omiecinski, C. J., Vanden Heuvel, J. P., Perdew, G. H., & Peters, J. M. (2010). Xenobiotic metabolism, disposition, and regulation by receptors: from biochemical phenomenon to predictors of major toxicities. *Toxicological Sciences*, *120*(suppl_1), S49-S75.
8. Shah, J. S., Shah, M. B., Goswami, S. S., & Santani, D. D. (2006). Mechanism of action of antiulcer activity of bark extracts of Manilkara hexandra against experimentally induced gastric ulcers in rats. *Pharmacognosy Magazine*, *2*(5), 46.
9. Badifu, G. I. O. & Ogunsua, A. O. (1991). Chemical composition of kernels from some species of Cucurbitaceae grown in Nigeria. *Plant Foods for Human Nutrition*, *41*(1), 35-44.
10. Oboh, G. (2005). Hepatoprotective property of ethanolic and aqueous extracts of fluted pumpkin (Telfairia occidentalis) leaves against garlic-induced oxidative stress. *Journal of medicinal food*, *8*(4), 560-563.
11. Hamzah, R. U., Jigam, A. A., Makun, H. A. & Egwim, E. C. (2013). Antioxidant properties of selected African vegetables, fruits and mushrooms: A review. *Licensee in Tech. Mycotoxin and Food Safety in Developing Countries*, 203-249.
12. Nwanna, E. E., & Oboh, G. (2007). Antioxidant and hepatoprotective properties of polyphenol extracts from Telfairia occidentaux (fluted pumpkin) leaves on acetaminophen induced liver damage. *Pakistan Journal of Biological Sciences*, *10*(16), 2682-2687.

13. Sujatha, K., Srilatha, C. H., Anjaneyulu, Y. & Amaravathi, P. (2011). Lead acetate induced nephrotoxicity in wistar albino rats, pathological, immunohistochemical and ultra structural studies. *Int. J. Pharm. Biol. Sci*, *2*(2), B459-B469.
14. Salman T.M., Luqman A.O., Wahab A.O. (2008). Aqueous extract of Telfairia occidentalis leaves reduces blood sugar and increases haematological and reproductive indices in male rats. *African Journal of Biotechnology*, 7(14), 2299-2303
15. Higgins, S. T., Silverman, K., & Heil, S. H. (Eds.). (2007). Contingency management in substance abuse treatment. *Guilford Press.*
16. Reitman, S., & Frankel, S. (1957). A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *American journal of clinical pathology*, *28*(1), 56-63.
17. Szasz, G. (1969). A kinetic photometric method for serum γ-glutamyl transpeptidase. *Clinical chemistry*, *15*(2), 124-136.
18. Englehardt, A. (1970). Measurement of alkaline phosphatase. AerzH labor,16 42 Evans, G. O. (Ed.). (1996). *Animal Clinical Chemistry: A Practical Handbook for Toxicologists and Biomedical Researchers*. CRC Press.
19. Gornall, A. G., Bardawill, C. J. & David, M. M. (1949). Determination of serum proteins by means of the biuret reaction. *Journal of biological chemistry*, *177*(2), 751-766.
20. Bucolo, G., & David, H. (1973). Quantitative determination of serum triglycerides by the use of enzymes. *Clinical chemistry*, *19*(5), 476-482.
21. Medina, M. J., Bartroli, J., Alonso, J., Blanco, M. & Fuentes, J. (1984). Direct determination of glucose in blood serum using Trinder's reaction. *Analytical letters*, *17*(5), 385-396.
22. Weatherburn, M. W. (1967). Phenol-hypochlorite reaction for determination of ammonia. *Analytical chemistry*, *39*(8), 971-974.
23. Henry, RF. (1974). Clinical Chemistry Principle and Techniques. 2nd edition, published by Harper and Row, New York.
24. Maruna, R. F. L., & Trinder, P. (1958). Analysis of serum sodium and potassium ions. *Clinical Chimica Acta*, *2*, 581-585.

25. Yakubu, M. T., Akanji, M. A., & Oladiji, A. T. (2007). Hematological evaluation in male albino rats following chronic administration of aqueous extract of Fadogia agrestis stem. *Pharmacognosy Magazine*, *3*(9), 34.
26. Bowman, W. C. & Rand, M. J. (1980). The blood: drugs affecting coagulation, fibrinolysis, haematopoiesis and functioning of blood cells. *Textbook of Pharmacology, WC Bowman, MJ Rand (eds). Oxford, London, Edinburgh, Melbourne, Blackwell Scientific Publication*, 21-15.
27. Adebayo, J. O., Adesokan, A. A., Olatunji, L. A., Buoro, D. O. & Soladoye, A. O. (2005). Effect of ethanolic extract of Bougainvillea spectabilis leaves on haematological and serum lipid variables in rats. *Journal of Biokemistri*, *17*(1), 45-50.
28. McLellan, E., MacQueen, K. M. & Neidig, J. L. (2003). Beyond the qualitative interview: Data preparation and transcription. *Field methods*, *15*(1), 63-84.
29. Ashafa, A.O.T., Grierson, D.S. & Afolayan, A.J. (2008). Composition and antibacterial activity of essential oil from Felicia muricata Thunb. Leaves. *J. Biol. Sci*., 8, 784-788.
30. Isnard, B.C., Deray, G., Baumelou A., Le Quintrec M. & Vanherweghem J.L. (2004). Herbs and the Kidney. *American Journal of Kidney Disases,* 44, (1) 1-11
31. Saad, B., Azaizeh, H., Abu-Hijleh, G. & Said, O. (2006). Safety of traditional Arab herbal medicine. *Evidence-Based Complementary and Alternative Medicine*, *3*(4), 433-439.
32. Rasekh, H. R., Nazari, P., Kamli-Nejad, M. & Hosseinzadeh, L. (2008). Acute and subchronic oral toxicity of Galega officinalis in rats. *Journal of ethnopharmacology*, *116*(1),21-26.
33. Evans, A.M. (1996). Membrane transport as a determinant of the hepatic elimination of drugs and metabolites. *Clin Exp Pharmacol Physiol*, 23(10-11); 970-4
34. Moudgil, K.D. & Narang, B.S. (1989). The liver and the biliary system. *Textbook of biochemistry and human biology. 2nd ed. New Delhi: Prentice-Hall of India Pvt Ltd.* 271-3.
35. Wurochekke, A. U., Anthony, A. E. & Obidah, W. (2008). Biochemical effects on the liver and kidney of rats administered aqueous stem bark extract of Xemenia Americana. *African Journal of Biotechnology*, *7*(16).

36. Panda, N.C. (1989). Kidney: In Textbook of Biochemistry and Human Biology (2nd edition). Talwer GP, Stivastava LM, Moudgil KD (eds). Prentice-Hall of India. Private Ltd. 276-292.
37. Nguyen, P., Leray, V., Diez, M., Serisier, S., Bloc'h, J. L., Siliart, B. & Dumon, H. (2008). Liver lipid metabolism. *Journal of animal physiology and animal nutrition*, *92*(3), 272-283.
38. Toyin, Y. M., Adewumi, A. M., & Temidayo, O. A. (2008). Alterations in serum lipid profile of male rats by oral administration of aqueous extract of Fadogia agrestis stem. *Research Journal of Medicinal Plant*, *2*(2), 66-73.
39. Panagiotakos, D. B. & Toutouzas, P. K. (2003). Importance of LDL/HDL cholesterol ratio as a predictor for coronary heart disease events in patients with heterozygous familial hypercholesterolaemia: a 15-year follow-up (1987-2002). *Current medical research and opinion*, *19*(2), 89-94.
40. Reddy, B. S., Rao, N. R., Vijeepallam, K. & Pandy, V. (2017). Phytochemical, pharmacological and biological profiles of Tragia species (family: Euphorbiaceae). *Africa Journal of Traditional, Complementary and Alternative Medicines*, *14*(3), 105-112.
41. Adewale, O. O., Samuel, E. S., Oluwuyi, O. O. & Akomolafe, S. F. (2018). Co-exposure of Lead Acetate and Sodium Arsenite Causes Alteration in the Markers of Liver and Kidney Functions in Male Wistar Rats. *Journal of Chemical, Environmental and Biological Engineering*, *2*(1), 32.