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Effect of extracts of *dialium guineense* stem bark on lipid peroxidation index and histological changes in kidneys of normal rats

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Abstract
Aim: To investigate the effect of extracts of *Dialium guineense* stem bark on lipid peroxidation index and histological changes in kidneys of normal rats.
Materials and Methods: Adult male Wistar rats (n = 70; 35 rats per extract) weighing 160 to 180 g were divided into seven (7) groups of five (5) rats each. Group I served as control, while rats in the other groups were administered varied doses of extract (200 - 5000 mg/kg body weight, bwt) orally for a duration of 28 days. Tissue malondialdehyde (MDA) level was measured as well as histopathological assessment.
Results: Percentage increases in body weights of rats treated with aqueous or ethanol extract of *D. guineense* stem bark were significantly reduced, when compared with control group ($p< 0.05$), but there were no significant differences in the corresponding relative organ weights and tissue MDA level among the groups ($p> 0.05$). Similarly, the extracts did not significantly alter the normal architecture of the kidney.
Conclusion: The aqueous and ethanol extracts of the medicinal plant did not elicit any deleterious effects on rat kidney.

Keywords: *Dialium guineense*, malondialdehyde, medicinal plant, nephrotoxicity, tissue histology

Introduction
The kidney is the key organ involved in whole-body homeostasis. It is a bean-shaped organ that regulates acid-base balance, electrolyte concentrations, extracellular fluid volume, and blood pressure. These functions are performed both independently and in conjunction with organs of the endocrine system [1]. Many of the kidney's functions are achieved via filtration, reabsorption, and secretion [2]. The nephron is the basic structural and functional unit of the kidney [2]. Filtration takes place in renal corpuscle. The kidney generates 180 L of filtrate a day, while reabsorbing a large percentage, allowing for the production of about 2 L of urine [3, 4]. As the main organ responsible for the elimination of unmodified drugs and metabolites, any alterations in kidney structure and function produce serious health consequences [5, 6]. Nephrotoxicity refers to injury to the kidneys or impairment of kidney function caused by exposure to xenobiotics including medicinal plants [7].
Herbal medicines derived from plant extracts are utilized to treat different clinical conditions. In recent years, increased attention has been paid to the protective effects of natural antioxidants against drug-induced toxicities especially those involving free radical generation [8, 9]. The World Health Organization (WHO) estimates that 80 percent of the population of some Asian and African countries presently use herbal medicine for some aspects of primary health care [10].
Medicinal plants have long been recognized as important sources of therapeutically active compounds [11]. Evidence-based research supports the medical and pharmacological benefits of plant-derived compounds with special interest in the identification and characterization of bioactive compounds from natural sources [12-14]. *Dialium guineense* is a medicinal plant used in Traditional Medicine for the treatment of different disease conditions, such as diarrhea, severe cough, bronchitis, wound, stomachaches, malaria, jaundice, ulcer and hemorrhoids [15-17]. Although the acute toxicity of its extracts have been reported, not much is known about the subchronic toxicity of the plant [18]. The aim of this study was to investigate the effect of extracts of *D. guineense* stem bark on lipid peroxidation index and histological changes in kidneys of normal rats.

Materials and Methods

Chemicals and Reagents

All reagents used in this study were of analytical grade and they were bought from British Drug House (BDH) (England), Merck (Germany) and Sigma-Aldrich Ltd. (USA).

Plant Material and Authentication

Fresh stem barks of *D. guineense* were obtained from Auchi, Edo State, Nigeria and authenticated at the herbarium of the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria (No. UBH_D330).

Plant Extraction

Extraction of the pulverized plant material was by maceration over a 72 h period [19]. A portion (500 g) of the powdered stem bark was soaked in 5000 mL distilled water or absolute ethanol. The resultant aqueous and ethanol extracts were filtered with a muslin cloth and freeze dried using a lyophilizer.

Experimental Rats

Adult male Wistar rats (n = 70; 35 rats per extract), which weighed between 160 and 180 g (mean weight = 170 ± 10 g) were purchased from the Department of Anatomy, University of Benin, Benin City, Nigeria. The rats were housed in metal cages under standard laboratory conditions: room temperature, 55-65% humidity and 12h light/12-h dark cycle. They were allowed free access to pelletized growers mash and clean drinking water. Prior to commencement of the study, the rats were acclimatized to the laboratory environment for seven days. Standard experimental procedures were followed for this study.

Experimental Design

The rats were divided into 7 groups (5 rats per group): Group I served as control, while rats in the other groups were administered varied doses of aqueous or ethanol extract (200 - 5000 mg/kg bwt) orally for a duration of 28 days. At the end of the 28th day the rats were fasted overnight and euthanized. The kidneys of all experimental rats were harvested, washed in ice-cold saline, blotted dry and placed in plain containers. Weighted portions of the organ were used to prepare 20% tissue homogenate. The tissue homogenate was subsequently centrifuged at 2000 rpm for 10 min to obtain supernatant, which was used for MDA determination.

Determination of lipid peroxidation in kidney

Malondialdehyde (MDA) level was measured in kidney homogenate [20].

Histological Examination of the Kidney

Sizeable portions of the kidney were sectioned and fixed in 10% formalin for 48 h, and thereafter dehydrated using varied concentrations of ethanol. Just before embedment in paraffin, the specimens were cleared thrice with xylene. Serial sections of 4 µm thickness were cut and stained with haematoxylin and eosin (H & E) according to standard protocol. Histopathological examination was carried out under light microscopy. In each H and E section, exactly 25 circular tubules were measured in two axes drawn perpendicular to each other with the aid of an image analyzer (Image Proplus, version 3.0).

Statistical Analysis

Count data are expressed as mean ± standard error of mean (n = 5). The statistical analysis was carried out using SPSS (version 20). The different groups were compared using Duncan multiple range test. Statistical significance was assumed at $p< 0.05$.

Results

Effect of Extracts of *D. guineense* Stem Bark on Weight Parameters

Percentage increases in body weights of rats treated with aqueous or ethanol extract of *D. guineense* stem bark were significantly reduced, when compared with control group ($p< 0.05$), but there were no significant differences in the corresponding relative organ weights among the groups ($p> 0.05$). These results are shown in Table 1.

Table 1: Percentage body weight increase and relative kidney weight of rats

Groups	% Increase in weight aqueous	Relative organ weight (x 10^{-2})	% Increase in weight ethanol	Relative organ weight (x 10^{-2})
Control	61.35 ± 4.11	3.34 ± 0.03	61.35 ± 4.11	3.34 ± 0.03
200 mg/kg bwt	49.09 ± 4.83[a]	3.59 ± 0.14	52.60 ± 2.92[a]	3.60 ± 0.08
500 mg/kg bwt	47.39 ± 3.09[a]	3.50 ± 0.11	22.63 ± 1.56[b]	3.87 ± 0.31
1000 mg/kg bwt	42.38 ± 2.61[a]	3.21 ± 0.07	21.00 ± 1.00[b]	3.30 ± 0.15
2000 mg/kg bwt	37.28 ± 3.94[a]	4.07 ± 0.62	18.30 ± 1.06[b]	3.47 ± 0.11
3500 mg/kg bwt	31.65 ± 2.83[b]	3.15 ± 0.11	17.73 ± 0.92[b]	3.84 ± 0.21
5000 mg/kg bwt	27.82 ± 0.40[b]	3.39 ± 0.31	16.80 ± 1.10[b]	3.50 ± 0.20

Data are percentage weight increase and relative kidney weight, and are expressed as mean ± SEM (n = 3)[a]. $p< 0.05$, when compared with control group; [b]$p< 0.05$, when compared with the other treatment groups.

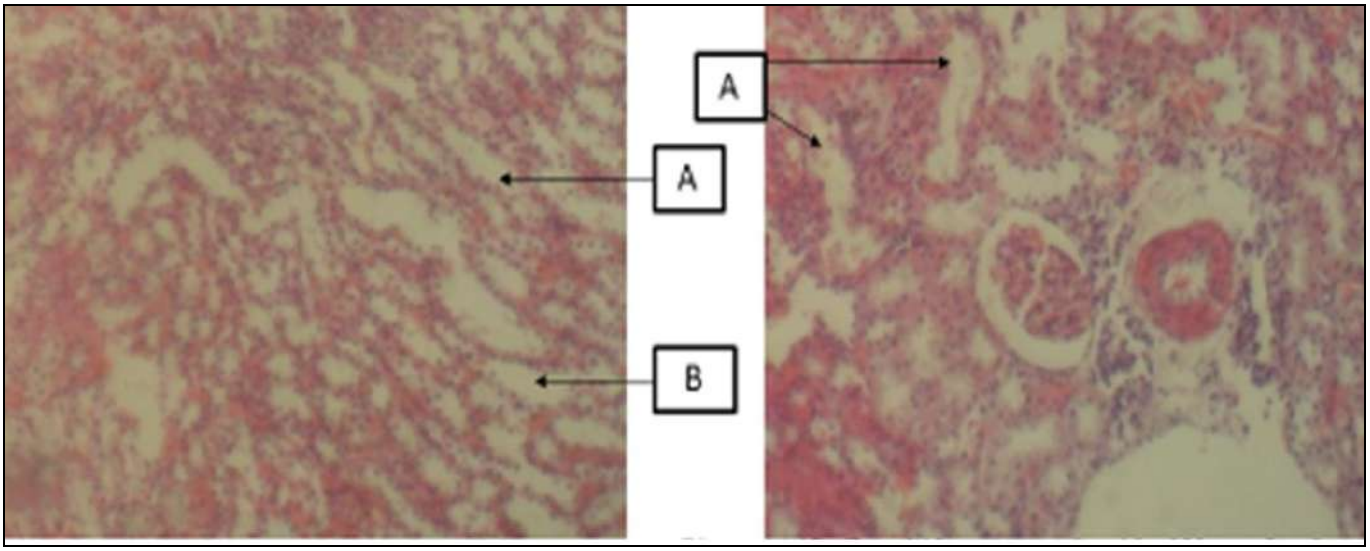
Effect of Extracts of *D. guineense* Stem Bark on MDA Level

The concentrations of MDA in kidney homogenates of rats treated with extracts of *D. guineense* stem bark were not significantly different from those of control group, except group 7 rats treated with 5000 mg/kg bwt of ethanol extract, which was significantly higher than that of control group ($p> 0.05$; Table 2).

Table 2: Concentrations of MDA in Kidney Homogenates

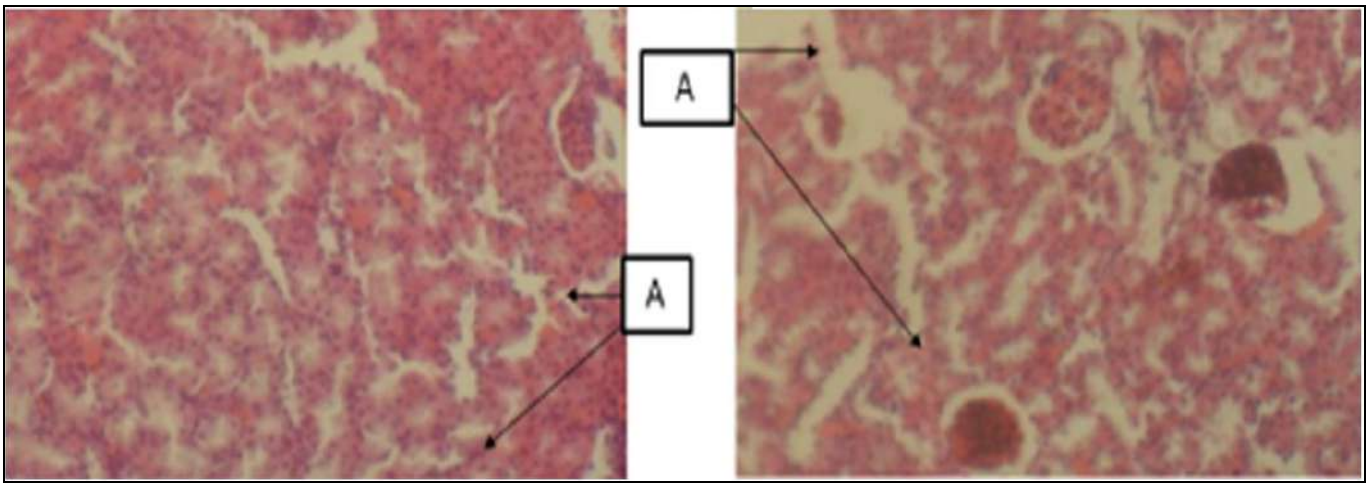
Groups	MDA Concentration (mole/mg protein) x 10^{-6}	
	Aqueous	Ethanol
Control	3.40 ± 1.00	3.40 ± 1.00
200 mg/kg bwt	4.01 ± 1.05	6.25 ± 1.74
500 mg/kg bwt	4.81 ± 1.50	5.96 ± 1.02
1000 mg/kg bwt	6.00 ± 1.40	4.93 ± 0.51
2000 mg/kg bwt	5.92 ± 0.51	6.49 ± 0.72
3500 mg/kg bwt	7.11 ± 1.50	7.01 ± 1.28
5000 mg/kg bwt	7.95 ± 0.91	9.14 ± 1.61[a]

Data are concentrations of kidney MDA and are expressed as mean ± SEM (n = 5). [a]p< 0.05, when compared with control group.



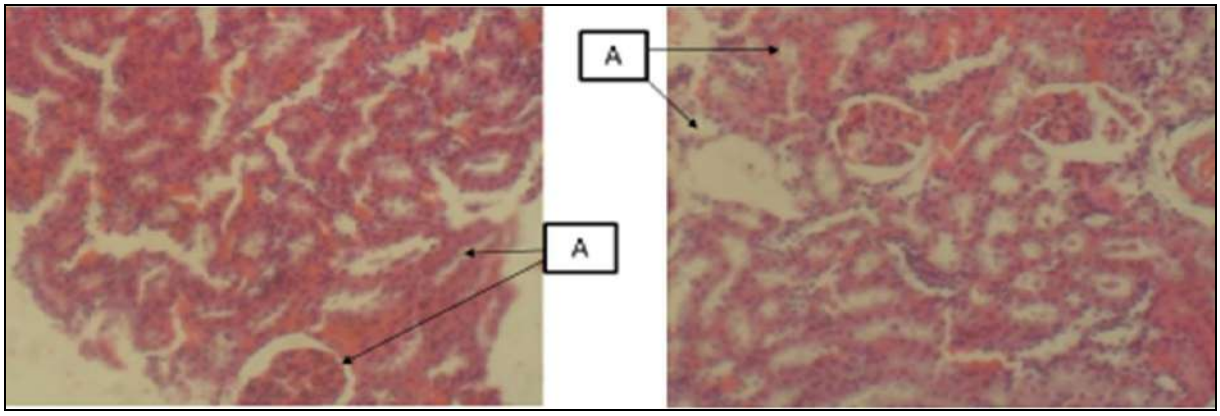
Plant 1: (Control) Rat kidney composed of A (tubules) and B (interstital space) (H & E x100)

Plant 2: Rat kidney treated with 200 mg/kg bwt aquesous extract of *D. guineense* showing A (normal renal architechture (H & E x 100)



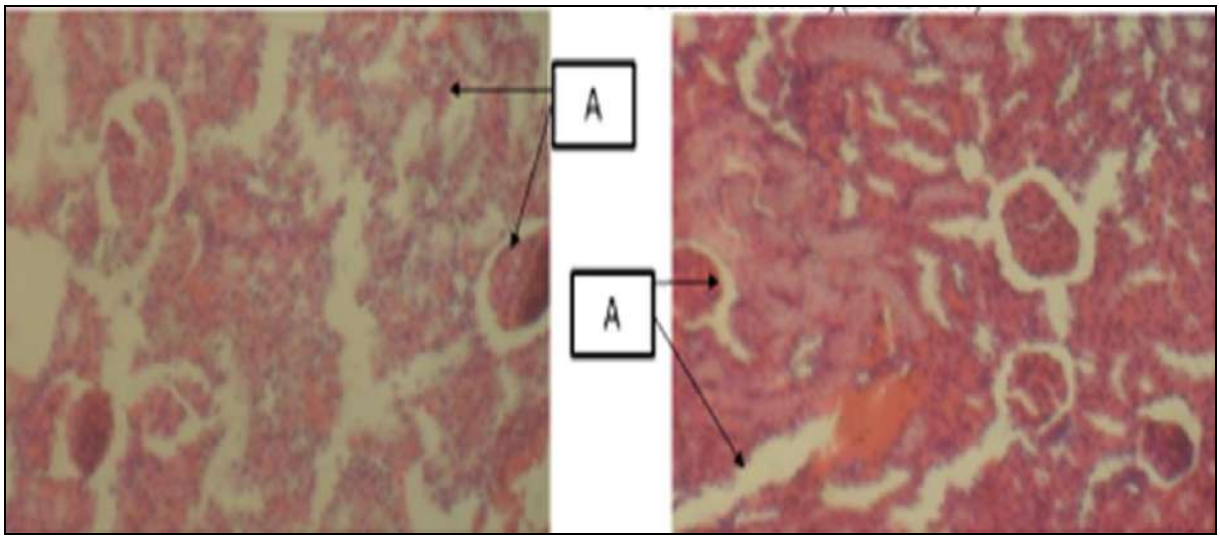
Plant 3: Rat kidney treated with 500 mg/kg bwt aqueous extract of *D. guineense* showing A (normal renal architecture) (H & E x100)

Plant 4: Rat kidney treated with 1000 mg/kg bwt aqueous extract of *D. guineense* showing A (normal renal architecture) (H & E x100)



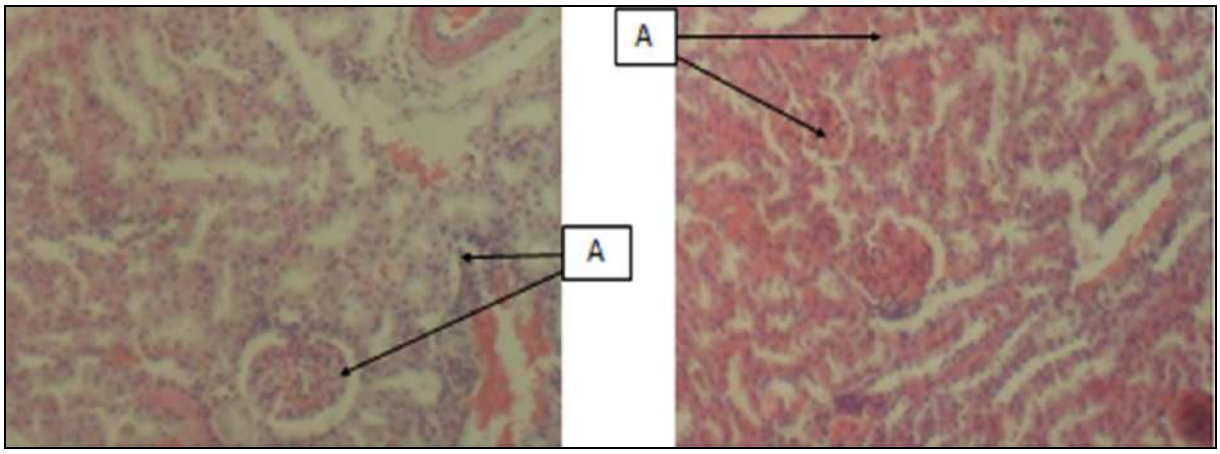
Plant 5: Rat kidney treated with 2000 mg/kg bwt aqueous extract of *D. guineense* showing A (normal renal architecture) (H & E x100)

Plant 6: Rat kidney treated with 3500 mg/kg bwt aqueous extract of *D. guineense* showing A (normal renal architecture) (H & E x100)



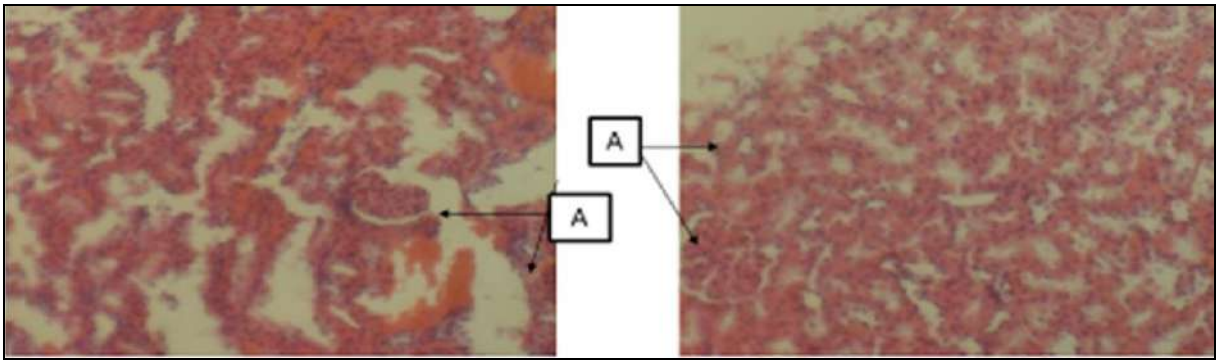
Plant 7: Rat kidney treated with 5000 mg/kg bwt aqueous extract of *D. guineense* showing A (normal renal architecture) (H & E x100)

Plant 8: Rat kidney treated with 200 mg/kg bwt ethanol extract of *D. guineense* showing A (normal renal architecture) (H & E x100)



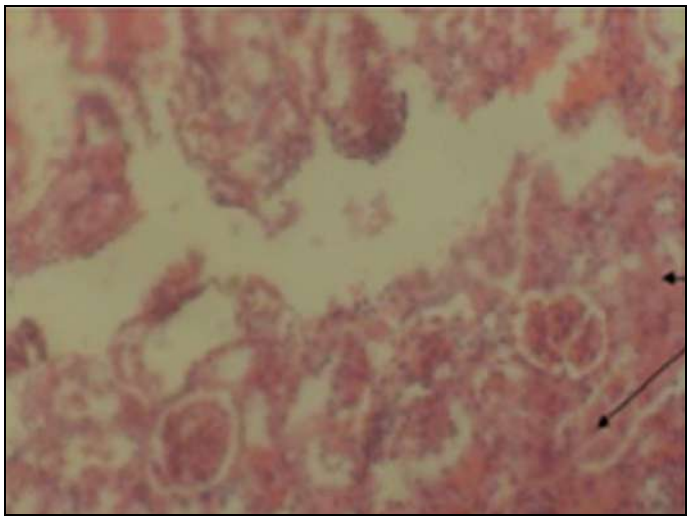
Plant 9: Rat kidney treated with 500 mg/kg bwt ethanol extract of *D. guineense* showing A (normal renal architecture) (H & E x100)

Plant 10: Rat kidney treated with 1000 mg/kg bwt ethanol extract of *D. guineense* showing A (normal renal architecture) (H & E x100)



Plant 11: Rat kidney treated with 2000 mg/kg bwt ethanol extract of *D. guineense* showing A (normal renal architecture) (H & E x100)

Plant 12: Rat kidney treated with 3500 mg/kg bwt ethanol extract of *D. guineense* showing A (normal renal architecture) (H & Ex100)



Plant 13: Rat kidney treated with 5000 mg/kg bwt ethanol extract of *D. guineense* showing A (normal renal architecture) (H & Ex100)

Discussion

A major setback limiting the use of plant extracts in modern medicine is the inability to ascertain safe dose. Many medicinal plants known to be effective against a number of diseases have also been shown to be toxic at certain doses and prolonged exposure [18]. Drugs continue to be taken off the market due to possible hepatorenal toxicity.

Due to the peculiarity of the functions carried out by the kidney, the organ is highly susceptible to injury from drugs and other substances. Several mechanisms are responsible for either inducing renal injury or worsening the damage process. The human body identifies almost all drugs as foreign substances (that is, xenobiotics) and subjects them to various chemical processes (metabolism) to make them suitable for elimination via the kidneys [1].

Statistics state that more than 100 kinds of herbal preparations can cause kidney damage [21]. Therefore, improving the understanding of herbal medicine formulations with nephrotoxic effects has become an urgent problem [21]. Different drugs can cause varied damage to the kidney. Hence, detection indicators are different and reflect the differentially damaged kidney parts. Among several mechanisms, induction of apoptosis in renal tubular epithelial cells, remains an important mechanism for subchronic nephrotoxicity.

Changes in body weight serve as a sensitive indicator of the general health status of animals. Weight loss often synonymous with loss of appetite is due to disturbances in carbohydrate, protein or fat metabolisms [22].

Histology, also known as microscopic anatomy or microanatomy, is the branch of Biology which studies the microscopic anatomy of biological tissues. It is the microscopic version of gross anatomy which looks at larger structures visible without a microscope. In Medicine, histopathology is the branch of histology that includes the microscopic identification and study of diseased tissue [23-25]. In kidney disease, several reports have documented gross and microscopic alterations of the morphology of renal tubules and nephrons [7]. This study investigated the effect of extracts of *D. guineense* stem bark on lipid peroxidation index and histological changes in kidneys of normal rats. The results showed that percentage increases in body weights of rats treated with aqueous or ethanol extract of *D. guineense* stem bark were significantly reduced, when compared with control group, but there were no significant differences in the corresponding relative organ weights and MDA levels among the groups. Similarly, the extracts did not significantly alter the normal architecture of the kidney. These results indicate that aqueous and ethanol extracts of *D. guineense* stem bark may be relatively safe, and agree with previous findings [26-30]. The protective capacity of extracts of the medicinal plant have been demonstrated [31-35].

Conclusion

The results of this study have demonstrated that the extracts of *D. guineense* stem bark are not deleterious to renal tubules and nephrons, and have provided a first time

evidence as to the relative safety of herbal formulations from the medicinal plant.

References

1. Walter F, Boron PHD. Medical Physiology: A Cellular and Molecular Approach. Elsevier/Saunders; c2004.
2. Clapp WL. Renal Anatomy. In: Zhou XJ, Laszik Cotran RS, Kumar V, Fausto N, Nelso F, Robbins SL, Abbas AK. Robbins and Cotran pathologic basis of disease (7th ed.). St. Louis, MO: Elsevier Saunders. 2005, 2009, 878.
3. Bard J, Vize PD, Woolf AS. The kidney: from normal development to congenital disease. Boston: Academic Press; c2003. p. 154.
4. Schrier RW, Berl T, Harbottle JA. Mechanism of the Antidiuretic Effect Associated with Interruption of Parasympathetic Pathways. Journal Clinical Investigation. 1972;51(10):2613-2620.
5. Cotran RS, Kumar V, Fausto N, Nelso F, Robbins SL, Abbas AK. Robbins and Cotran pathologic basis of disease (7th ed.). St. Louis, MO: Elsevier Saunders, 2005, 878.
6. Le T. First Aid for the USMLE Step 1. New York: McGraw-Hill Medical; c2013.
7. Abu OD, Imafidon KE, Obayuwana HO. Nephrotoxic and *in vivo* antioxidant effects of *citrullus lanatus* seed extract. Biomedical Journal of Science and Technical Research. 2021;33(5):26281-26286.
8. Rai MK. Herbal medicines in India; retrospect and prospect. Fitoterapia. 1994;65(6):483-491.
9. Stickel F, Schuppan D. Herbal medicine in the treatment of liver diseases. Dig Liver Dis. 2007;39(4):293-304.
10. World Health Organization (WHO). ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure 2008. European Journal of Heart Failure. 2012;14(8):803-869.
11. Superling F. Introduction to Toxicity Evaluation Session. Environmental Health Perspective. 1979;32:259.
12. Walum E. Acute Oral Toxicity. Environmental Health Perspectives. 1998;106(2):497-503.
13. Handa SS, Khanuja SPS, Longo G, Rakesh DD. Extraction technologies for medicinal and aromatic plants. International Centre for Science and High Technology, Trieste. Italy, 2008, 21-25.
14. Sandoval M, Okuhama NN, Zhang XJ, Condezo LA, Lao J, Angeles FM. Anti-inflammation and antioxidant activities of cat's claw (*Uncaria tomentos* and *Uncaria guinensis*) are independent of their alkaloid content. Phytomedicine. 2002;9(4):325-337.
15. Dalziel JM, Hutchison J. Flora of West Tropical Africa. Vol.1 (2nd Ed). The White friars Press Ltd. London, 1973, 561.
16. Bero J, Ganfon H, Jonville MC, Frederich M, Gbaguidi F, De MP, *et al*. *In vitro* antiplasmodial activity of plants used in Benin in traditional medicine to treat malaria. Journal of Ethnopharmacology. 2009;122(3)**:**439-444.
17. Arogba SS, Ajiboro A, Odukwe I. A physicochemical study of Nigerianvelvet tamarind (*Dialium guineense* L.) fruit. Journal of the Science of Food and Agriculture. 2006;66(4**):**533-534.
18. Abu OD, Onoagbe IO. Acute toxicity of aqueous and ethanol extracts of *Dialium guineense* stem bark. Journal of Bioinnovation. 2021;10(2):427-432.
19. Abu OD, Imafidon KE, Iribhogbe ME. Biochemical effect of aqueous leaf extract of *Icacina trichanta* Oliv. on urea, creatinine and kidney oxidative status in CCl_4-induced Wistar rats. Nigerian Journal of Life Sciences. 2015;5(1):85-89.
20. Guttridge JMC, Wilkins C. Cancer dependent hydroxyl radical damage to ascorbic acid. Formation of thiobarbituric acid reactive product. FEBS Lett. 1982;137(2):327-340.
21. Huang W, Liu C, Xie L, Wang Y, Xu Y, Li Y. Integrated network pharmacology and targeted metabolomics to reveal the mechanism of nephrotoxicity of triptolide. *Toxicol.* Res. (*Camb*). 2019;8(6):850-861.
22. Eaton DL, Klaassen CD. Principle of Toxicology, in Klassen CD (ed) Casaret and Doll's Toxicology. The Basic Science of Poisons. 6th edition. McGraw-Hill, 2001, 11-13.
23. Rosai J. Why microscopy will remain a cornerstone of surgical pathology. Lab Invest. 2007;87(5):403-408.
24. Titford M, Bowman B. What May the Future Hold for Histotechnologists? Laboratory Medicine. 2012;43(2):e5-e10.
25. Wick MR. The hematoxylin and eosin stain in anatomic pathology: An often-neglected focus of quality assurance in the laboratory. Seminars in Diagnostic Pathology. 2019;36(5):303-311.
26. Abu OD, Orobator ON, Momodu IB. Evaluation of the Effect of Total Saponins and Tannins Isolated from *Dialium guineense* Stem Bark on CCl_4 - Induced Hepatotoxicity in Wistar Rats. Global Journal of Medical and Clinical Case Reports. 2022;9(3):035-038.
27. Abu OD, Ogbebor EO, Omage JI. Effect of Extracts of *Dialium guineense* Stem Bark on Oxidative Status in Rats Exposed to CCl_4. Journal of Clinical Gastroenterology and Hepatology. 2022;4(3):124-127.
28. Abu OD, Onoagbe IO, Ekugum E. Hepatotoxicity of Graded Doses of Ethanol Extract of *Dialium guineense* Stem Bark in Wistar Rats. Journal of Pharmaceutical and Bio-Medical Sciences. 2022a;2(9):347-352.
29. Abu OD, Onoagbe IO, Ekugum E. Nephrotoxic Evaluation of Aqueous Stem Bark Extract *of Dialium guineense* in Normal Wistar Rats. Journal of Pharmaceutical and Bio-Medical Sciences. 2022b;2(9):353-357.
30. Abu OD, Okuo AV, Osemwota OF. Total Saponins and Tannins of *Dialium guineense* Stem Bark Protect Against CCl_4-induced Oxidative Stress in Rats Liver. International Journal of Medical and Clinical Case Reports. 2022;1(1):15-20.
31. Abu OD, Umar AB, Ajuwa OI. Protective Property of Total Saponins and Tannins of *Dialium guineense* Stem Bark in CCl_4-Induced Cardiotoxicity in Rats. World Journal of Genetics and Molecular Biology. 2022;1(1):1-6.
32. Abu OD, Ezike TV, Ajuwa OI. Cardioprotective property of extracts of *Dialium guineense* stem bark in rats exposed to CCl_4. American Journal of Biomedical Science and Research, 2022, 689-693.
33. Abu OD, Iyare HE, Ogboi KU. Antioxidant Property of Total Saponins and Tannins of *Dialium guineense* Stem

Bark in Rats Hearts Exposed to CCl_4. Journal of Clinical Epidemiology and Toxicology. 2022;3(3):1-4.
34. Abu OD, Omage JI, Ogbebor EO. Effect of Total Saponins and Tannins Isolated from the Stem Bark of *Dialium guineense* on Lipid Profile and CCl_4- Induced Histological Changes in Liver of Wistar Rats. Journal of Medicine and Biology. 2022;3(2):1-9.
35. Abu OD, Okuo AV, Ayele PE. Pancreatotoxic. Effect of Aqueous Extract of *Dialium guineense* Stem Bark in Wistar Rats. International Journal of Novel Research in Life Sciences. 2022;9(5):31-37.
36. Abu OD, Alegun O, Ojo AU. Pancreatotoxicity of Ethanol Extract *of Dialium guineense* Stem Bark in Rats. World Journal of Pharmaceutical and Life Sciences. 2022;8(11):40-45.