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Nephroprotective effect of *Pterocarpus mildbraedii* leaf extract in paracetamol-induced toxicity in Wistar rats: A histopathological investigation

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Abstract

Pterocarpus mildbraedii leaf is among the most commonly consumed leafy vegetables in west African sub-region. In South-East and South-South geopolitical zones of Nigeria, it is used for treatment of various health disorder such as headaches, pains, fever, convulsions, and respiratory disorders. This study was aimed at evaluating the possible effect of ethanol leaf extract of *Pterocarpus mildbraedii* on paracetamol-induced nephrotoxicity in Wistar rats. Twenty (20) Wistar rats weighing between 190 – 240 g were used for this study and divided into five (5) groups, each group having four (4) animals, described as follows; Group A was given distilled water only (positive control). Group B was given 700 mg/kg paracetamol with no treatment (negative control); Group C was given 700 mg/kg paracetamol and treated with 60 mg/kg of plant extract. Group D was given 700 mg/kg paracetamol and treated with 30 mg/kg of plant extract. Group E given 700 mg/kg of paracetamol and treated with 15 mg/kg of plant extract. At the end of the four (4) weeks of treatment, the kidneys were removed, weighed and sacrificed for histological examinations. Coronal sections of the kidney tissues were immersion fixed in 10 % PBS-buffered formalin and embedded in paraffin. The results obtained revealed a normal cellular architecture in the groups treated with the leaf extract. Conclusively, the result suggests protective effects of ethanol extract of *P. mildbraedii* leaf on the paracetamol-induced toxicity on the kidney cells.

Keywords: *Pterocarpus mildbraedii*; Medicinal plants; Nephroprotective; Histopathology; Paracetamol

1. Introduction

Paracetamol (acetaminophen) is one of the widely used analgesic and antipyretic drugs worldwide, and in most countries, it is usually available over the counter without prescription [1, 2], resulting in its abuse, which further causes toxicity to various organs such as the liver and the kidney [3]. Paracetamol is the active metabolite of phenacetin and acetanilide, both are popular analgesic and antipyretics in their own rights [4]. Its overdose is known to cause hepatotoxicity [1, 2, 5], and if significant, it can trigger nephrotoxicity [2, 5]. Clinically and histologically, paracetamol toxicity creates acute tubular necrosis, which is one of the major causes of acute renal damage [6]. However, these toxicities may be attributed to the metabolism of the paracetamol through glucuronidation and sulfation in the liver, while water soluble metabolites are excreted through the kidney [2]. Oxidative stress has also been attributed to be the main mechanism of paracetamol toxicity [7].

The kidney is a vital organ and it plays a vital role in the excretion of diverse waste products produced by metabolism into the urine. It processes the blood supplied to it through filtration, reabsorption, secretion and excretion (which the

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end-product is urine) [8]. The kidney participates in whole-body homeostasis, regulating acid-base balance, electrolyte concentrations, extracellular fluid volume, and blood pressure. The kidney accomplishes these homeostatic functions both independently and in concert with other organs, particularly those of the endocrine system [9].

Kidney failure is an increasingly common condition with limited treatment options that is causing a major financial and emotional burden on individuals and the community at large [10]. Globally, diseases of the kidneys are amongst the most important causes of death and disability recently [11, 12]. Kidney failure prompts a slow and advanced deterioration of kidney function enhanced by various factors including infections, auto immune diseases, etc. [13]. Nevertheless, drug-induced nephrotoxicity which can cause kidney damage can be relieved by medicinal plants, because of their dietary nature and less adverse reactions [7]. One of such medicinal plants is *Pterocarpus mildbraedii.*

Pterocarpus mildbraedii belongs to the family Leguminosae. It is found in Sierra Leone, Liberia, Cote d'ivoire, Ghana, Benin, Cameroon, Gabon, Nigeria and Tanzania [14]. In some part of Eastern Nigeria, the young and tender leaves of this plant are used traditionally as vegetable for the preparation of soups [15, 16]. Biological activities of *Pterocarpus mildbraedii* has been reported; anti-inflammatory [17, 18], anti-diabetic, antioxidant [18, 19, 20, 21], hepatoprotective [22], thrombolytic [18], nephroprotective [22], cardioprotective [23], and antimicrobial [24]. Phytochemical screening showed that the *Pterocarpus mildbraedii* leaves contain alkaloids, flavonoids, tannins, steroids, glycosides, terpenoids, phlobatannins and saponins [21, 24, 25, 26]. Its leaves have been found to contain vital mineral elements such as calcium, sodium, potassium, phosphorus, iron, magnesium, zinc, manganese, copper and chromium [21]. The Igbo tribe of Eastern Nigeria and some tribes in Southern Nigeria use the leaf extracts in the treatment of headaches, pains, fever, convulsions, and respiratory disorders and as antimicrobial agents [21, 27]. This research is geared towards investigating the possible histopathological changes induced by *Pterocarpus mildraedii* leaves extract on the kidney to ascertain if the extract has any adverse or protective properties on the organ following its varying dose and the doses of paracetamol administered.

2. Material and methods

2.1. Plants material

The fresh leaves of *P. mildbraedii* were locally harvested from a garden located at 4 Miles in Calabar Municipal Local Government Area of Cross River State, Nigeria. The leaves were identified and authenticated by a taxonomist in the Department of Botany, Cross River University of Technology where the voucher specimen (CRUTECH No. 112) was deposited in the herbarium. The leaves were separated from the stalk, washed and air-dried at room temperature for two (2) weeks, after which the leaves were then blended to powder form.

2.2. Extraction of plant material

Five hundred (500) g of the powdered leaf was macerated in two (2) litres of 97 % ethanol. The extraction was carried out with the use of Soxhlet extraction. The filtrate from the extraction was concentrated by evaporating the excess ethanol to obtain thick slurry. The dry-crystalline extract was kept in a refrigerator.

2.3. Experimental animals

Twenty (20) Wistar rats weighing between 190- 250 g were used for this study. The animals were housed in well-ventilated cages and kept in the animal house of the Department of Human Anatomy, Cross River University of Technology. The environmental condition for the animals was suitable, with good ventilation and was always cleaned and disinfected, the cages were also cleaned as well as beddings changed regularly. The rats were acclimatized for two (2) weeks. The animals were fed with commercially manufactured Top Feeds Grower's mash (Premier Feed Mills Co Ltd., Nigeria) and water *ad libitum* for the period of the experiment (28 days).

2.4. Experimental design

Twenty (20) Wistar rats were grouped into five (5) groups, each group having four (4) animals as follows; Group A was given distilled water only (positive control); Group B was given 700 mg/kg of paracetamol (negative control); Group C was given 700 mg/kg of paracetamol and treated with 60 mg/kg of *Pterocarpus mildbraedii* ethanol extract; Group D was given 700 mg/kg of paracetamol and treated with 30 mg/kg of *Pterocarpus mildbraedii* ethanol extract; and Group E was given 700 mg/kg of paracetamol and treated with 15 mg/kg of *Pterocarpus mildbraedii* ethanol extract.

2.5. Drug procurement, preparation and administration

Paracetamol (Emzor Pharmaceuticals Industries Ltd., Lagos-Nigeria) was purchased from Adonah Pharmacy, Okuku, Yala Local Government Area, Cross River State. The paracetamol tablets were dissolved in distilled water and administered orally by a daily dose of 700 mg/kg per body weight to groups B, C, D, and E followed by the oral administration of *Pterocarpus mildbraedii* leaves extract daily concomitantly to groups C, D, and E, for a period of twenty-eight (28) days. At the end of the treatment period, the animals were sacrificed, the kidneys were removed and weighed immediately for histological examination. Coronal sections of the kidney tissues were immersion fixed in 10 % PBS-buffered formalin and embedded in paraffin.

2.6. Histological studies

Histological sections of the kidney were stained with Haematoxylin and Eosin (HE). Each was evaluated with an Olympus light microscopy (Olympus, Tokoyo, Japan) with high resolution digital camera system. For electron microscopic examination, small blocks of kidneys were fixed at 4 % glutaraldehyde, post fixed in 2 % osmium tetraoxide, dehydrated in graded ethanol and then embedded in epoxy resin. Ultrathin sections were stained with lead citrate and uranyl acetate and examined using an electronic microscopy (Jem-1230, JEol, Japan).

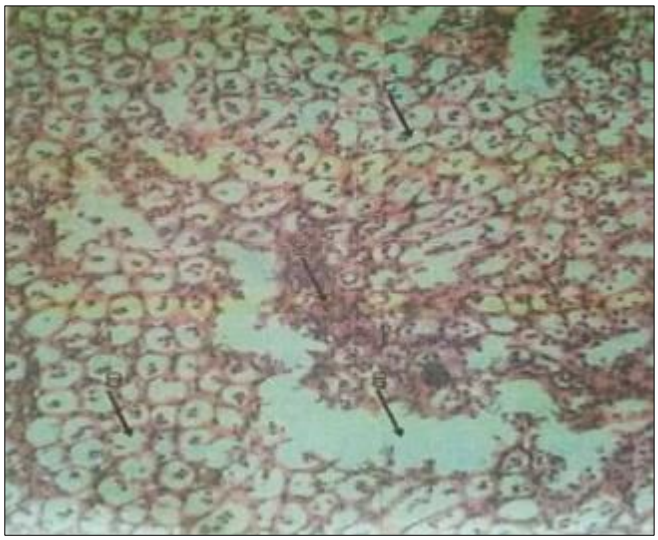
3. Results and discussion

Paracetamol is widely used as pain reliever with some harmful side effects (if administered in excess) when completely metabolized in the body. N-acetyl-p-benzoquinoneimine (NAPQ) is a typical harmful metabolite produced by paracetamol which damages cells in the kidney leading to kidney failure [28]. Nephropathy is widely encountered among people in the world's population irrespective of the age, race, environment and geographical variability. It occurs as a result of disturbance in renal function due to various drug interactions and chemicals [29]. The kidney injuries induced by analgesics are chronic interstitial nephritis and renal papillary necrosis which results from the decreased blood flow to the kidney, rapid consumption of the antioxidant and subsequent oxidative damage to the kidney. Renal toxicity in paracetamol poisoning has been attributed to cytochrome p-450 mixed function oxidase isoenzymes present in the kidney and the role of prostaglandin synthetase and N-deacetylase enzymes [30]. Toxicity of paracetamol can cause hepatic necrosis, nephrotoxicity, extra hepatic lesions and even death in humans and experimental animals [31]. However, severity of paracetamol toxicity varies depending on the doses and whether appropriate treatment is received. Histologically, it has been proven with evidence that toxicity of paracetamol with the most adverse effect of overdose is necrosis [32].

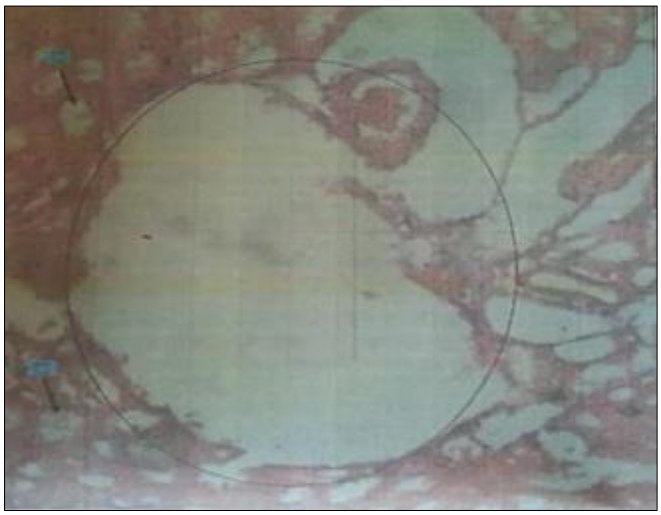
The kidney plays a vital role in the excretion of urine and maintaining homeostasis of water and electrolyte concentration in the body [33]. Pronephros, mesonephros, and metanephros are the embryological kidney in the different stages of kidney development with the pronephros as the most immature form of kidney and the metanephros as the most developed [34]. As the fetus develops, the torso elongates and the kidneys rotate and migrate upward within the abdomen which causes the length of the ureter to increase. Any alteration or abnormality associated with the kidneys could lead to non-performance or inefficiency in carrying out these functions by the kidney. The abnormalities associated with kidney function could be ascertained by evaluating the histological examination of the organ among others [35].

Histologically from this study, the kidney photomicrograph showed the cortex with prominent glomerulus and closely packed renal tubules in the normal control group (group A). However, aggregation of proximal convoluted tubules (CT) with their brush borders thereby, giving the tissue parenchyma a spongy appearance. The collecting duct (CD) was seen with adjourning peritubular capillaries (PC). No pathology was observed or seen therefore, this conforms with a normal typical histological appearance of the kidney (Fig. 1A). The photomicrograph of group B shows few distal convoluted tubules with a central area of focal tubular necrosis (encircled area), a mononuclear inflammation. Likewise, the kidney shows variable degrees of distortion of the cortex, the mesangium is loosely packed and there is loss of glomerular architecture (Fig. 1B).

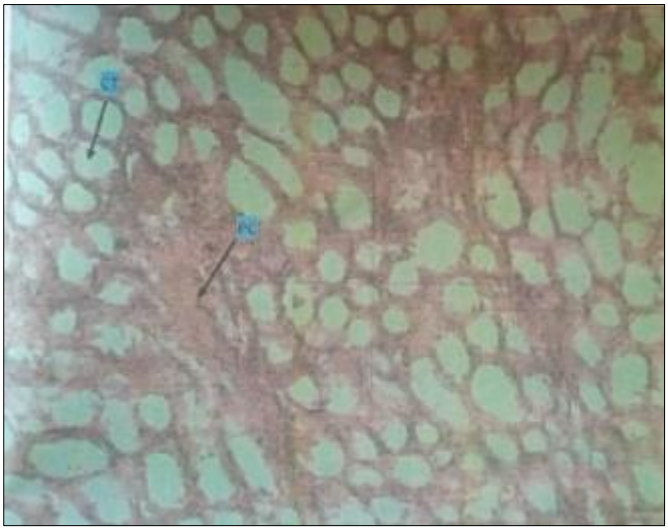
The kidney section shows the collecting tubules (CT), convoluted tubules indicating an insignificant tubulointerstitial distortions in group C. Also, a urinary/corpuscular space (CP) can also be seen. Therefore, no pathology is observed (Fig. 1C). More so, it was showed that numerous convoluted tubules (CT), peritubular capillaries were also adjourning the tubules in group D (Fig. 1D). While in group E, convoluted tubules (CT) and peritubular capillaries were seen interspersing them. Some glomeruli were also seen with their vascular pole (VP), indicating no pathology (Fig. 1E).



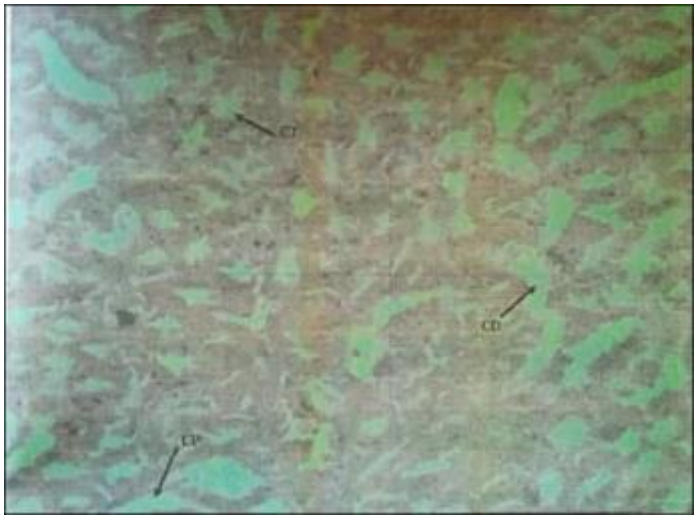
(A)



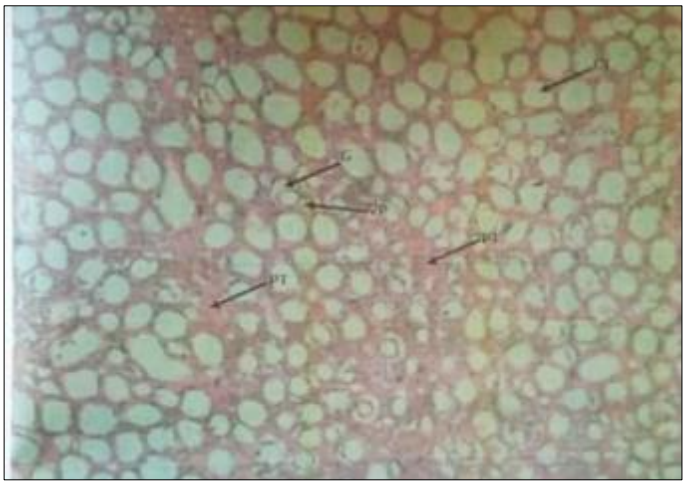
(B)



(C)



(D)



(E)

Figure 1 Photomicrograph showing the kidney at 28 days. H&E stain × 400. A: Normal control, B: 700 mg/kg paracetamol (negative control); C: 700 mg/kg paracetamol and 60 mg/kg *Pterocarpus mildbraedii* ethanol extract; D: 700 mg/kg paracetamol and 30 mg/kg *Pterocarpus mildbraedii* ethanol extract; and E: 700 mg/kg paracetamol and 15 mg/kg *Pterocarpus mildbraedii* ethanol extract

This ameliorative effect in the deterioration induced by paracetamol can be attributed to the presence of phenolic compounds in the studied plant. Phenolic compounds are known to have antioxidant properties, which plays an essential role in protecting the kidney from oxidative stress induced by paracetamol [26]. In validation of this finding, several studies (*in vivo* and *in vitro*) have been carried out confirming the antioxidant potentials of *Pterocarpus mildraedii* [18, 20, 21]. However, a similar finding was obtained by Canayakin *et al*. [2] and Ezekwesili *et al*. [36].

4. Conclusion

This study suggests that *Pterocarpus mildbraedii* leaf extract can pose a nephroprotective effect at a low and moderate dose. However, if administered in high dose, nephrotoxicity can occur. Therefore, the ameliorative effect on the kidney is in a dose dependent manner.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare that there is no conflict of interests.

Statement of ethical approval

The research study was carried out according to the guidelines approved by CRUTECH Institutional Research Ethical Committee (IREC) following the principle laid down in the Declaration of Helsinki (1964), as revised in 2013 and National Institute of Health (NIH) Principles of Laboratory Animal Care. No human participants were involved in the study.

References

[1] Adil M, Kandhare AD, Ghosh P, Venkata S, Raygude KS, Bodhankar SL. Ameliorative effect of naringin in acetaminophen-induced hepatic and renal toxicity in laboratory rats: role of FXR and KIM-1. Renal Failure, 2016; 38(6): 1007-1020.

[2] Canayakin D, Bayir Y, Baygutalp NK, Karaoglan ES, Atmaca HT, Ozgeris FBK, Keles MS, Halici Z. Paracetamol-induced nephrotoxicity and oxidative stress in rats: the protective role of *Nigella sativa*. Pharmaceutical Biology, 2016; 54(10): 2082-2091.

[3] Odinga T, Eka E. Hepatoprotective and nephroprotective potency of *Ricinodendron heudelotii* against acetaminophen-induced toxicity in Wistar albino rats. Research Journal of Medicinal Plants, 2020; 14: 167-173.

[4] Graham GG, Davies MJ, Day RO, Mohamudally A, Scott KF. The modern pharmacology of paracetamol: therapeutic actions, mechanism of action, metabolism, toxicity and recent pharmacological findings. Inflammopharmacology, 2013; 21(3): 201–232.

[5] Ali FU, Ominyi MC, Nwankwo OVU, Ibiam UA, Ogbanshi ME. Comparative effects of ethanolic extract of *Gongronema latifolium* and *Piper guineense* on blood electrolytes in ethanol exposed Wistar rats. Biochemistry and Analytical Biochemistry, 2015; 4: 179.

[6] Blantz RC. Acetaminophen: acute and chronic effects on renal function. American Journal of Kidney Disease, 1996; 28(1): S3–S6.

[7] Eraky SM, Abo El-Magd NF. Omega-3 fatty acids protect against acetaminophen-induced hepatic and renal toxicity in rats through HO-1-Nrf2-BACH1 pathway. Archives of Biochemistry and Biophysics, 2020; 687: 108387.

[8] Mescher AL. Junqueira's Basic Histology, 14th edition. Lange. 2016; p. 393.

[9] Glodny B, Unterholzner V, Taferner B. Normal kidney size and its influencing factors – a 64-slice MDCT study of 1.040 asymptomatic patients. Biochemistry and Medical Council of Urology, 2009; 9(1): 19.

[10] Singh P, Srivastava MM, Khemani LD. Renoprotective effects of *Andrographis paniculata* (Burm. f.) Nees in rats. Upsala Journal of Medical Sciences, 2009; 114(3): 136-139.

[11] Mascha V, Rashmi K, Priyadarshi SS. Comparing age wise reference intervals for serum creatinine concentration in a “reality check” of the recommended cut-off. Indian Journal of Clinical Biochemistry, 2006; 21: 90-94.

[12] Draczevski L, Teixeira ML. Avaliação do perfil bioquímico e parâmetros hematológicos em pacientes submetidos à hemodiálise. Review Saud Pesq, 2011; 4(1): 15-22.

[13] Eduardo OC, Kaue A, Idania AA. Influence of hemodialysis on the plasma concentration of adenosine deaminase in patients with chronic kidney disease. Jornal Brasileiro de Patologia e Medicina Laboratorial, 2015; 51: 153-157.

[14] Bosch CH. *Pterocarpus mildraedii* Harms. Plant Resources of Tropical Africa, 2004; 3: 78-80.

[15] Chinma CE, Igyor MA. Micronutrients and anti-nutrient content of selected tropical vegetables grown in South East, Nigeria. Nigerian Food Journal, 2007; 25(1): 111-116.

[16] Adegbite AV, Ezekwesili CN. Effects of ethanol and aqueous leaf extracts of *Pterocarpus mildbraedii* on hematology parameters and cholesterol in albino rats. African Journal of Biochemistry Research, 2017; 11(6): 28-33.

[17] Otuechere AC, Farombi EO. *Pterocarpus mildbraedii* (Harms) extract resolves propanil-induced hepatic injury via repression of inflammatory stress responses in Wistar rats. Journal of Food Biochemistry, 2020; 44(12): e13506.

[18] Fajobi AO, Emma-Okon BO, Oyedapo OO. In vitro antioxidant, anti-inflammatory and thrombolytic properties of leaf extract of *Pterocarpus mildbraedii* harms. Ife Journal of Science, 2020a; 22(3): 065-074.

[19] Omale J, Okafor NO, Omede A. Polyphenol content cytotoxicity and antioxidant activities of some selected Nigerian vegetable food. International Journal of Chemical Science, 2008; 6(4): 1714-1725.

[20] Ifesan BOT, Egbewole OO, Ifesan, BT. Effect of fermentation and nutritional composition of selected commonly consumed green leafy vegetables in Nigeria. International Journal of Applied Sciences and Biotechnology, 2014; 2(3): 291- 297.

[21] Usunomena U, Igwe VC. Phytochemical screening, mineral composition and *in vitro* antioxidant activities of *Pterocarpus mildbraedii* leaves. International Journal of Scientific World, 2016; 4(1): 23-26.

[22] Akinwunmi KF, Amadi CV, Awodiran MO. The effect of aqueous leaf extracts of *Pterocarpus mildbraedii* on crude oil-induced toxicity in Albino rats. Journal of Herbs, Spices and Medicinal Plants, 2021; 27(4): 365-376.

[23] Fajobi AO, Emma-Okon BO, Oyedapo OO, Dare CA, Akinlalu AO, Komolafe IJ, Ogunsusi M. Cardioprotective activities of *Pterocarpus mildbraedii* leaves on isoproterenol-induced myocardial infarction in rats. European Journal of Medicinal Plants, 2020b; 31(10): 20-37.

[24] Abare-Jen L, Mensah JK, Boroqouye SL. Evaluation of the phytochemistry, antimicrobial and antioxidant efficacy of the stem bark extracts of *Pterocarpus mildbraedii*. IOSR Journal of Applied Chemistry (IOSR-JAC), 2020; 13(5): 15-20.

[25] Amadi ES, Oguoma OI, Ibekwe VI, Abanobi SE, Chikwendu CI, Egbadon OE. Prevalence and the effect of plant extracts on community associated methicillin resistant *Staphylococcus aureus* in Owerri, Imo State, Nigeria. Journal of Research in Biology, 2013; 3(4): 967- 976.

[26] Uchegbu RI, Iwuoha GU, Elenwoke UE, Ibe CO, Amanze KO. Identification of phytochemicals present in the leaves of *Pterocarpus mildbraedii* (Harms) by GC/MS analysis. IOSR Journal of Applied Chemistry (IOSR-JAC), 2015; 8(7): 06-10.

[27] Ogukwe CE, Oguzie EE, Unaegbu C, Okolue BN. Phytochemical screening of the leaves of *Sanseviera trifasciata*. Journal of Chemical Society of Nigeria, 2004; 29(1): 8-9.

[28] El-Shaibany A, Al-Habori M, Al-Massarani S, El- Gamal A, Al-Ajami A, Al-Adhl A. Hepatoprotective effect of *Pandanus odoratissimus* L inflorescence extracts in acetaminophen-treated Guinea pigs. Tropical Journal of Pharmaceutical Research, 2016; 15(2): 259–267.

[29] Watkins PB, Kaplowitz N, Slattery JT, Colonese CR, Colucci SV, Stewart PW, Harris SC. Aminotransferase elevations in healthy adults receiving 4 grams of acetaminophen daily: A randomized controlled trial. JAMA, 2006; 296(1): 87-93.

[30] Mazer M, Perrone J. Acetaminophen-induced nephrotoxicity: pathophysiology, clinical manifestations, and management. Journal of Medical Toxicology, 2008; 4(1): 2–6.

[31] Ray SD, Mumaw VR, Raje RR, Fariss MW. Protection of acetaminophen-induced hepatocellular apoptosis and necrosis by cholesteryl hemisuccinate pretreatment. Journal of Pharmacology and Experimental Therapeutics, 1996; 279(3): 1470–1483.

[32] Blakely P, McDonald BR. Acute renal failure due to acetaminophen ingestion: a case report and review of the literature. Journal of American Society of Nephrology, 1995; 6(1): 48–53.

[33] Ross P, Willson T. Anatomy and physiology in health and illness (9th ed). Sidney Churchill Livingstone. 2001; pp. 340.

[34] Bruce MC. Human embryology and development biology (3rd ed). Saint Louis; Mosby. 2004. pp 79.

[35] Imo C, Arowora KA, Ezeonu CS, Yakubu OE, Nwokwu CD, Azubuike NC, Sallah YG. Effects of ethanolic extracts of leaf, seed and fruit of *Datura metel* L. on kidney function of male albino rats. Journal of Traditional and Complementary Medicine, 2019; 9: 271-277.

[36] Ezekwesili CN, Adegbite AV, Okani OC. Effects of leaf extracts of *Pterocarpus mildbraedii* on renal and heart functions of rats. Animals Research International, 2016; 13(2): 2446-2453.