Global Scientific JOURNALS

GSJ: Volume 9, Issue 2, February 2021, Online: ISSN 2320-9186
www.globalscientificjournal.com

Effect of ethanol leaf extract of *Terminalia chebula* extract on kidney of wister rats

Sabastine Aliyu Zubairu[1], Joseph Oyepata Simeon[2], Joseph Opeyemi Tosin[3]

1 Department of Pharmacology and Therapeutics, Faculty of Pharmacy, Gombe State University, Gombe State, Nigeria

[2]Department of Pharmacology, Faculty of Pharmaceutical Sciences, Federal University, Oye Ekiti State, Nigeria.

[3]Department of Pharmacy, University College Hospital, Ibadan, Oyo State, Nigeria

Author to whom correspondence should be addressed

Sabastine Aliyu Zubairu

and

Joseph O. S

Abstract

Introduction/Aim: Nephrotoxicity is toxicity in the kidneys. It is a poisonous effect of some substances, both toxic chemicals and medications, on kidney function. Toxicity tests are intended to evaluate the toxicity of the chemical after repeated administration and also to help in establishing doses for the longer-term. *Terminalia chebula*, commonly known as black- or chebulic myrobalan, is a species of Terminalia, widely used in South Asia from India and Nepal east to southwest China (Yunnan), and south to Sri Lanka, Malaysia, and across Africa. It is widely used in management of various illment. This work is aim at evaluating the toxic effect of Terminalia chebula on kidney of wister rats.

Method: rats of either sex were selected. Group 1 received distilled water (10 ml/kg), while group 2, 3 and 4 received Terminalia Chebula 200, 400 and 800 mg/kg respectively. Animals were kept in standard cages and given access to the extract, water and food orally for 28 days, after which they were weighed and sacrificed. Blood was collected by cardiac puncture and

taken immediately for hematological and chemopathological analysis. The kidney was also harvested for histological study on the effect of the extract using haematotoxylin and eosin (H&E) staining technique.

Result: There was Significant (P<0.05) decrease in RBC, HGB, MCV, while there was no significant change in the level of neutrophiles, basophiles, eosinophiles and platelets. Terminalia chebula caused slightly significant (p<0.05) increased in Na level at 50 mg/kg and Creatinine level at 50 mg/kg dose levels respectively when compared to the control when compared to the control. Other parameters (K, CL and Urea levels) were not significantly affected. Histological study reveals slight tubular distortion.

Conclusion: The result of the study showed that at normal dose the plant may have no effect on the kidney which suggests that the plant may be nephrologically safe for consumption.

Keyword: Terminalia chebula, rat, blood, kidney

Introduction

Across the cultures, knowledge about use of medicinal plants exists in the form of local folklore available with families, tribes and cultures, handed down from generation to generation[1]. Medicinal plants or their extracts have been used by humans since time immemorial for different ailments and have provided valuable drugs such as analgesics (morphine), antitussives (codeine), antihypertensives (reserpine), cardiotonics (digoxin), antineoplastics (vinblastine and taxol) and antimalarials (quinine and artemisinin)[2]. Medicinal plant drug discovery continues to provide new and important leads against various pharmacological targets including cancer, malaria, cardiovascular diseases and neurological disorders[3]. Plants have proven to be a novel source for bioacive natural products. They have evolved and adapted over millions of years to withstand bacteria, insects, fungi and weather to produce unique, structurally diverse secondary metabolites. Their ethnopharmacological properties have been used as a primary source of medicines for early drug discovery[4].

Nephrotoxicity is one of the most common kidney problems and occurs when your body is exposed to a drug or toxin that causes damage to your kidneys[5]. When kidney damage occurs, it will be unable to rid of the body of excess urine, and wastes[6]. The blood electrolytes (such as potassium, and magnesium) will all become elevated. Nephrotoxicity can be temporary with a temporary elevation of lab values (BUN and/or creatinine). If these levels are elevated, these may be due to a temporary condition such as dehydration or you may be developing renal (kidney failure). If the cause of the increased BUN and/or creatinine levels is determined early,

and healthcare provider implements the appropriate intervention, permanent kidney problems may be avoided. Modern medicine has used extracts since at least the 1950s as a laxative[7,8]. If accidentally ingested by infants, it can cause side effects such as severe diaper rash[9]. The active ingredients has several senna glycosides which interact with immune cells in the colon.

Terminalia chebula tree is about 50-80 feet tall in height[10]. It has round crown and spreading branches. The bark is dark brown with some longitudinal cracks. Leaves are ovate and elliptical, with two large glands at the top of the petiole. The flowers are monoecious, dull white to yellow, with a strong unpleasant odour, borne in terminal spikes or short panicles. T. chebula is found in the Sub Himalayan tracks from Ravi eastwards to West Bengal and Assam, ascending up to the altitude of 1500 m in the Himalayas. This tree is wild in forests of Northern India, central provinces and Benga[11,12]. The tree is also spread across Africa. The fruit is mild laxative, stomachic, tonic, alterative, antispasmodic[13]. It is useful in ophthalmic, hemorrhoids, dental caries, bleeding gums, ulcered oral cavity. Its paste with water is found to be antiinflammatory, analgesic and having purifying and healing capacity for wounds. Its decoction is used as gargle in oral ulcers, sore throat. Its powder is a good astringent dentifrice in loose gums, bleeding and ulceration in gums[14]. It is good to increase appetite, digestive aid, liver stimulant, stomachic, gastrointestinal prokinetic agent, and mild laxative[14]. The powder of T. chebula fruits has been used in chronic diarrhea. It is used in nervous weakness, nervous irritability. It promotes the receiving power of five senses[15]. It is adjuvant in hemorrhages due to its astringent nature and good for chronic cough, chorizo, sore throat as well as asthma. Terminalia chebula is the main ingredient in the Ayurvedic formulation Triphala which is used for kidney and liver dysfunctions. The dried fruit is also used in Ayurveda as a purported antitussive, cardiotonic, homeostatic, diuretic, and laxative[16]. The aim of this work is to evaluate the effect of ethanol extract of Terminalia chebula on the kidney of rats.

Materials and Method

Animals Male and female wister rats were obtained from Bingham University, Animal House. They were maintained on standard animal pellets and given water ad libitum. Permission and approval for animal studies were obtained from the College of Health Sciences Animal Ethics Committee of Bingham University.

Plant collection: Leaves of Terminalia chebula were collected from its natural habitat from nearby Karu village, Nasarawa State, Nigeria. The plant was authenticated from Department of Botany, Bingham University, Nasarawa State Nigeria.

Plant extraction: The leaves were shadow dried for two weeks. The dried plant material was

further reduced into small pieces and pulverized. The powdered material was macerated in 70% ethanol. The liquid filtrates were concentrated and evaporated to dryness at 40 C° in vacuum using rotary evaporator..

Animal study: Twenty four (24) rats of either sex (125-250g) were selected and randomized into four groups of six rats per group. Group 1 served as the control and received normal saline (10ml/kg) while the rats in groups 2, 3 and 4 were giving 200, 400, and 800 mg/kg of extract respectively. The weights of the rats were recorded at the beginning of the experiment and at weekly intervals. The first day of dosing was taken as D0 while the day of sacrifice was designated as D29.

Haematological analysis: The rats were sacrificed on the 29th day of experiment. Blood samples were collected via cardiac puncture. One portion of the blood was collected into sample bottles containing EDTA for hematological analysis such as Hemoglobin concentration, white blood cell counts (WBC), differentials (neutrophils, eosinophils, basophils, lymphocyte and monocyte), red blood cell count (RBC), platelets and hemoglobin (Hb) concentration using automated Haematology machine (Cell-Dyn, Abbott, USA).

Kidney Function Test: The following biochemical parameters were assayed as markers of kidney function using diagnostic kits; Level of electrolytes (Na+, K+, Cl-, and HCO3-), creatinine and blood urea. The above parameters were determined at the Chemical Pathology Department of University of Jos Teaching Hospital. Kidney harvested were preserved in 10% formal saline solution, processed, sectioned and stained with Heamatoxylin and eosin (H&E) according to standard procedures at Department of Chemical Pathology, University of Jos Teaching Hospital, Jos.

Statistical analysis: Data were expressed as the Mean ±Standard Error of the Mean (SEM). Data were analyzed statistically using one-way Analysis of Variance (ANOVA) followed by Dunnett's post hoc test for multiple comparisons between the control and treated groups. Values of $P \leq 0.05$ were considered significant.

Result

Effect of sub-acute oral administration of Terminalia chebula on hematological parameters in rats.

Terminalia chebula caused significant ($p<0.05$) decrease in the level of red blood cell, hemoglobin, platelet etc. andsignificantly ($p<0.05$) caused an increase in mean corpuscular hemoglobin concentration in the rats at the dose level of 400 mg/kg compared to the control. The

level of basophiles, neutrophiles, eosinophils and lymphocytes were however not significantly ($p<0.05$) affected by mean corpuscular hemoglobin concentration (Table 1).

Effect of 28 days oral administration of Terminalia chebula on renal indices and electrolytes in Wistar rats.

Terminalia chebula slightly significant ($p<0.05$) increased Na and creatinine level at 200 mg/kg when compared to the control. Other parameters (K, CL, and Urea levels) were not significantly affected.

Histopathological Investigations of the effect of 28 days oral administration of Terminalia chebula on renal indices and electrolytes in Wistar rats.

The kidney showed very slight tubular distortion and glomerular necrosis at 50 mg/kg. There was also, Slight tubular necrosis with lymphocyte hyperplasia at 100 mg/kg. Normal renal histological features were observed in the control group.

Table 1: Effect of 28 days oral administration of ethanol leaf extract of Terminalia chebula on hematological parameters in wistar rats.

		Treatment (mg/kg)		
Hematological parameters	DW(10ml/kg)	200 mg/kg	400 mg/kg	800 mg/kg
WBC ($\times10^9$/L)	9.166±0.772	7.640±1.429	4.700±0.556*	8.230±1.088
RBC ($\times10^{12}$/L)	9.23±0.32	9.65±0.67	7.11±0.75*	7.81±0.22
HGB (g/dL)	15.56±0.56	15.45±0.88	12.33±0.76*	15.58±0.37
HCT (g/dL)	57.18±2.03	57.60±3.75	35.67±3.18*	54.40±1.82
MCV (fL)	66.45±0.93	64.40±1.14	57.77±0.31*	69.61±1.73

MCH (pg)	19.17±0.17	17.80±1.02	18.83±0.37	18.80±0.20
MCHC (g/dL)	29.17±0.17	27.40±1.12	32.50±0.62*	27.60±0.68
PLT ($\times10^9$/L)	620.83±52.81	567.00±96.41	252.00±50.38*	670.40±55.72
LYM (%)	86.83±4.06	85.00±4.18	82.83±5.89	86.40±3.14
NEUT ($\times10^9$/L)	11.83±3.68	11.83±3.58	14.40±5.20	13.20±3.11
EOSI ($\times10^9$/L)	1.53±0.34	1.40±0.76	1.90±0.22	1.40±0.43
BASO ($\times10^9$/L)	1.10±0.28	2.45±0.43	2.50±1.50	3.40±2.23

Data presented as Mean ± SEM: n = 6, One way ANOVA, followed by Dunnett's post hoc for multiple comparison *significantly different from the distilled water (DW) control at $p<0.05$. DW = distilled water

(WBC = white blood cells, RBC = red blood cells, HGB = hemoglobin, HCT = hematocrit, MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, PLT = platelet, LYM = lymphocyte, NEUT = neutrophils, EOSI = eosinophils, BASO = basophils).

Table 2: Effect of 28 days oral administration ethanol leaf extract Terminalia chebula on renal indices and electrolytes in wistar rats.

		Treatment (mg/kg)		
Renal indices and electrolytes	DW(10ml/kg)	200 mg/kg	400 mg/kg	800 mg/kg
Potassium (mmol/L)	6.30±0.21	7.11±0.69	5.62±0.38	5.78±0.15
Sodium (mmol/L)	136.00±1.90	142.33±2.02	149.00±1.97*	140.25±1.31
Chloride (mmol/L)	114.00±5.77	98.80±6.41	105.20±1.18	110.50±1.66
Urea (mmol/L)	9.56±0.29	9.42±0.60	9.11±0.20	8.67±0.36

Creatinine (µmol/L)	65.40±9.13	74.41±12.12*	64.82±16.11	69.70±5.25

Data presented as Mean ± SEM: n = 6, One Way ANOVA, followed by Dunnett's post hoc for multiple comparison *significantly different from the distilled water (DW) control at $p < 0.05$. DW = distilled water.

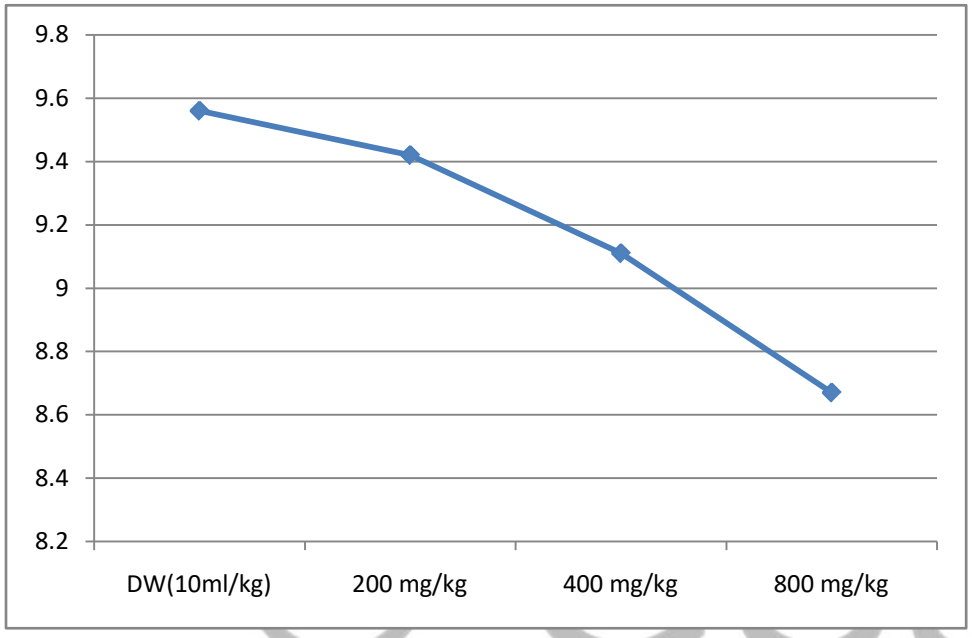

Figure 1: Effect of *Terminalia chebula* on level of serum urea level

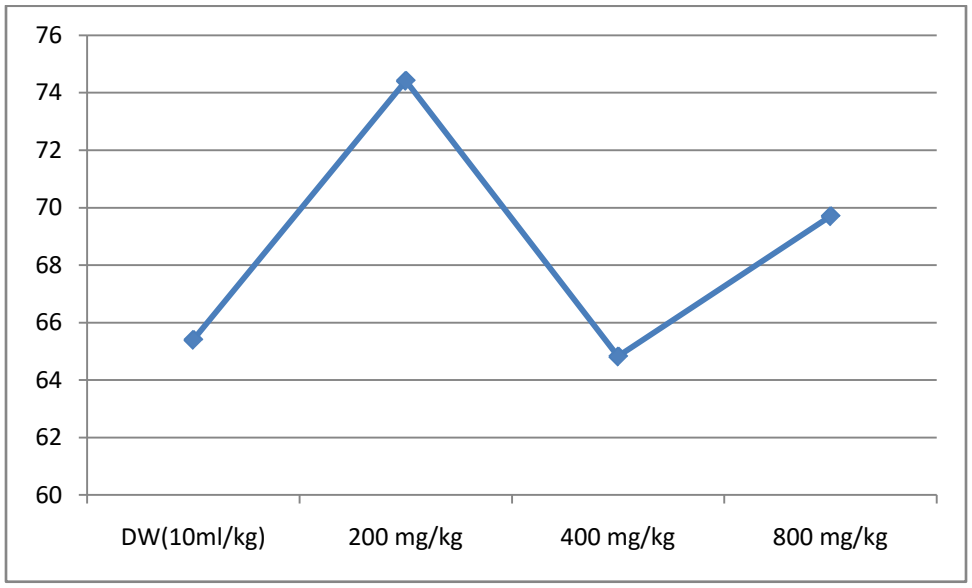

Figure 2: Effect of *Terminalia chebula* on level of serum creatinine level

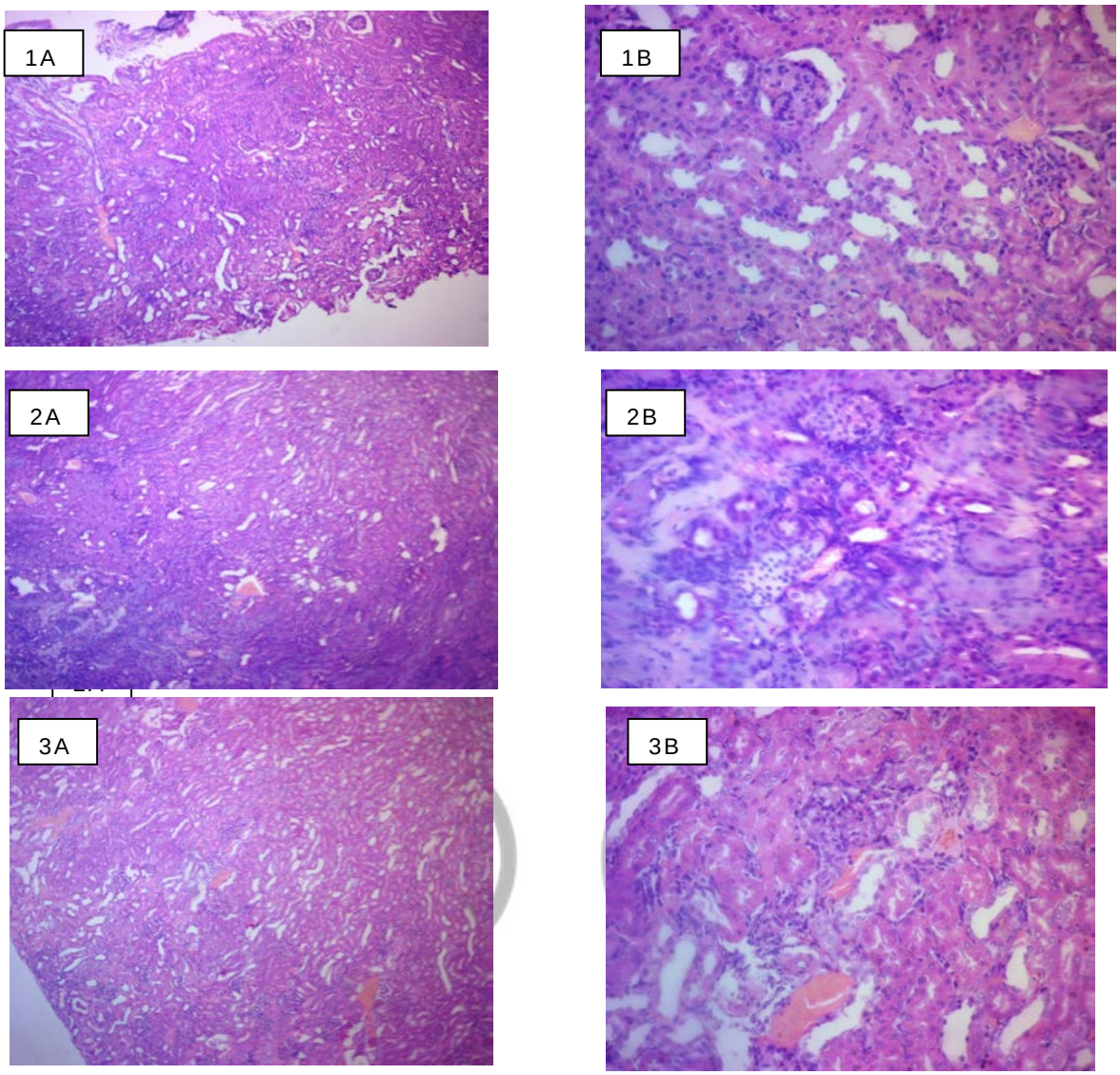

Figure 4: Histological sections of Kidneys of rats treated with Normal saline 10 ml/kg (1), *Terminalia chebula* 200 mg/kg (2), *Terminalia chebula* 200 mg/kg bw (3) and *Terminalia chebula* 400 mg/kg at magnification A (x100) and B(x400)) stained with H&E Technique.

Discussion

Traditionally, plants and plant extracts were used to cure many diseases and disorders. However, before usage it is of utmost important to ensure its safety[17,18]. The extract may be therapeutically very efficient but if its toxicity assessment is not worked out, it will not be accepted. Hence, toxicity assessment of plants with proven therapeutic use is of utmost important[19]. Toxicity data are required to predict the safety associated before the use of medical products[20,21]. There have been reports of accidental medicinal plant poisoning and over dose. In most cases this traditionally formulated drugs are consumed without appropriately establishing the dose that is safe for use. This has resulted into many untoward after effect[23]. Hematological parameters are

useful indices that can be employed to assess the toxic potentials of plant extracts in living systems[24]. They can also be used to explain blood relating functions of chemical compound/plant extract. Present result showed that ethanol leaf extract of Terminalia chebula caused a reduction in the level of red blood cells, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration which means that it can significantly reduce oxygen carrying capacity of the blood and thus cause anemia[6,25]. Anemia is a condition where the blood has insufficient red blood cells to carry oxygen from the lungs. to the rest of the body or not enough hemoglobin, the iron-rich protein that carries oxygen inside the red blood cells and gives blood its red color[26]. Anemia takes several forms and may vary in severity and duration[19]. Also reductions in packed cell volume (PCV) and red blood cell (RBC) were also observed in rats administered with the extract. This implies that Terminalia chebula could cause disturbances in osmoregulatory system of the blood cells and/or oxidative injury to the cell membrane. The extract could suppress the haemopoietic system. The reduction may have also occurred due to lysis of blood cells. Sule et al,[20,26] also observed decrease in RBC, PCV, hemoglobin andlymphocytes in rats fed with extracts of Acalypha wilkesiana. The major functions of the white blood cell and its differentials are to fight infections, defend the body by phagocytocis against invasion by foreign organisms and to produce or at least transport and distribute antibodies in immune response[26]. The extract had no effect on white blood cell parameters, suggesting that it has no effect on the immune cells and the immune system.

An increase creatinine level can be observe in some kidney diseases, due to loss of normal excretory function of the creatinine, when there is a muscular cells damage or following an incompatable medication interfering with the normal functioning of the kidney[27]. Creatinine, is mostly derived from endogenous sources by tissue creatinine breakdown[12,23]. The serum creatinine concentration of the group that received 200 mg/kg of the extract was slightly higher than the control group. Atangwho et al.[22] reported elevated serum creatinine level as an indicator of possible kidney dysfunction. Gross et al.[18] in a study indicated that a rise in serum creatinine level could suggest a possible damage to the functioning nephrons of the kidney. The measurement of creatinine concentration in serum was a useful index for the diagnosis of chronic kidney disease and when serum creatinine level was higher than the normal value, renal failure was most likely a possible outcome[28]. Increased serum creatinine concentration has been considered a marker of assessing nephrotoxicity as reported by Anwar et al.[10] and Ali et al[17]. it is also possible that at high dose the antioxidant activity of Terminalia chebula becomes conspicuous, negating and possibly providing protective property to cells. In this study, serum

urea was unaffected suggesting that the plant may cause slight damage to the kidney. Thus serum urea concentration is often considered a more reliable renal function predictor than serum creatinine[28,29]. The histopathological analysis, showed that in all groups after 28 days administration of ethanol extract of Terminalia chebula the kidney there was slight changes at the cellular level in comparison to control. This resonates with other parameters that the leaves of the plant slightly have nephrotoxic effect over a long period of time.

Conclusion: Result from the study suggests that the plant possesses may have no toxic consequences on the kidney and the urinary system. Further study is needed to evaluate its nephrprotective activity.

Acknowledgement: The authors wish to thank everyone who has contributed to the success of this research work.

Reference

1. Aniszewski, T. (2007) Alkaloids—Secrets of Life. In Alkaloid Chemistry, Biological Significance, Applications and Ecological Role; Elsevier Science: Amsterdam, The Netherlands; p. 334.

2. Lemli, J (1995), Mechanism of action of sennosides, Bulletin de l'Académie Nationale de Médecine, 179 (8): 1605–11.

3. Arif, T., Bhosale, J. D., Kumar, N., Mandal, T. K., Bendre, R.S., Lavekar, G.S. and Dabur, R. (2009) Natural products – antifungal agents derived from plants. Journal of Asian Natural Products Research 11 (7): 621–638.

4. Kinnunen, O., Winblad, I., Koistinen, P., Salokannel, J (1993), Safety and efficacy of a bulk laxative containing senna versus lactulose in the treatment of chronic constipation in geriatric patients, Pharmacology. 47 Suppl 1: 253–5.

5. Balunas, M. J. and Kinghorn, A. (2005) Drug discovery from medicinal plants. Life Sciences 78(5): 31-41.

6. Duke, James A. (2002). Handbook of medicinal herbs. Duke, James A., 1929- (2nd ed.). Boca Raton, Florida: CRC Press. ISBN 978-0849312847

7. Abyar, Selda; Khandar, Ali Akbar; Salehi, Roya; Abolfazl Hosseini-Yazdi, Seyed; Alizadeh, Effat; Mahkam, Mehrdad; Jamalpoor, Amer; White, Jonathan M.; Shojaei, Motahhareh; Aizpurua-Olaizola, O.; Masereeuw, Rosalinde (2019). "In vitro nephrotoxicity and anticancer potency of newly synthesized cadmium complexes". Scientific Reports. 9 (1): 14686.

8. Galley HF (2000). "Can acute renal failure be prevented". J R Coll Surg Edinb. 45 (1): 44–50. PMID 10815380. Archived from the original on 2005-10-18.

9. Naesens M, Kuypers DR, Sarwal M (2009). "Calcineurin inhibitor nephrotoxicity". Clin. J. Am. Soc. Nephrol. 4 (2): 481–509.

10. Joseph O. S., Modupe B., Wazis C. H., Joseph O. T., Sabastine A. Z., Musa T. L. and Moh'd A. S. (2019). Effect of administration ethanol leaf extract of terminalia chebula on liver of wister rat. International Journal of Research and Scientific Innovation. Volume VI (Issue VII). Page 91- 97.

11. Joseph O. S., Builders M., Wazis C. H., Sabastine A. Z., Musa T. L. and Joseph O. T. (2019). Histological study of effect of ethanol stem extracts of Homalium letestui on thioacetamide - induced injury in albino rat, using various staining techniques. International Journal of Research and Scientific Innovation. Volume VI (Issue VII). Page 77 – 85.

12. Sabastine A. Z., Musa T. L., Joseph O. S., Builders M. and Joseph Opeyemi T. (2019). Histological study of effect of ethanol stem extracts of Homalium letestui in paracetamol induced injury in albino rat, using various staining techniques. American Journal of Biomedical Science & Research. 4(2). Page 82 – 89.

13. Joseph O.S., Builders M., Joseph O. T, Ariahu E. C., Zubairu S. A., Musa T. and Oyepata P.J. (2019). Toxicity study of ethanol leaf extract of ocimum canum on heart and lipid profile of wister rats. International Journal of Current Advanced Research. Volume 8. (Issue 05). Page 18800 – 18803.

14. Samson A. O., Joseph O. S., Samson O. A. and Emem R. A. (2019). Effect of Citrus Linton Juice and Tamoxifen on The oxidative activities of MCT-7 cell induced Bresat Cancer in Sprawgue Dawley Rats. Saudi Journal of Biomedical Research. Volume 8 (7). Page 76-92.

15. Joseph O. S, Builders M., Isinkaye D. R., Sebastine A. Z., Musa T., Oyepata J. P., Joseph O. T. and Wazis C. (2019). Sub-Acute Toxicity Study of Ethanol Leaf Extract of Terminalia chebula On Brain, Stomach and Spleen of Wister Rats. American Journal of Biomedical Science & Research. 3(3). Page 277-282.

16. Joseph O.S, Builders M., Joseph O, T. , Zubairu S. A., Musa T. And Oyepata P. J (2019). Sub-Acute Toxicity Study of Ethanol Leaf Extract of Ocimum Canum on Liver of Wister Rats. International Journal of Research and Scientific Innovation. Volume VI (V). Pp. 364-369.

17. Oyebadejo S. A, Joseph O. S, Adesite S. O and Omorilewa A.O. (2019). Effect of Citrus Limon Juice and Tamoxifen on the Tumour growth mass Indices, Cell Proliferation, Cell Viability and Cytogenetic (Mitotic Index) of Sprague Dawley Rats Induced MCF-7 Breast Cancer Cells. Saudi Journal of Biomedical Research. (4). Pg. 216 - 225.

18. Modupe I. B., Oyepata S. J. and Akpobome R. V. (2019). Effect of Parkia biglobosa extract on open skin wound healing in dexamethasone - induced hyperglycaemia and histological assessment in rats. African Journal of Pharmacy and Pharmacology. Vol. 13(8), pp. 84-89.

19. Gupta SS. (1994). Prospects and perspectives of natural plant products in medicine. Indian J Pharmacol.;2:1–12.

20. Recknagel RO, Glende EA. Jr. Carbon tetrachloride Hepatotoxicity: An Example of Lethal Cleavage. Critical Reviews in Toxicology; 1973. 2, 263-297. [20]. Doi, K., Kurabe, S.,Shimazu,N. and Inagaki, M. (1991). Systemic Histopathology of Rats with CCl4-Induced Hepatic Cirrhosis, Laboratory Animals, 25,21-25.

21. Dorman, H. J., Kosar, M., Karlos, K., Holm. Y. and Hittuner, R. (2003). Antioxidant Properties and Composition of Aqueous Extracts From Menthaspecies, Hybrids, Varieties and Cultivars. Journal of Agriculture Food and Chemistry, 51, 4563– 4569.

22. Wazis C.H., Joseph O.S., Modupe B, Joseph O.P. (2020). Effect of ethanol leaf extract of cassia angustifolia extract on heart and lipid profile of wister rats. African Journal of Pharmaceutical Research & Development. Vol. 12 No.1. Page 1-8.

23. Wazis C.H., Joseph O.S., Modupe B, Joseph O.P. (2020). Toxicological Study of the Effect of Ethanol Leaf Extract of Pterocarpus santalinus Extract on Liver of Wister Rats. Nigeria biomedical Journal. Accepted for publication. Page 17-29.

24. Wazis C.H., Joseph O.S., Modupe B, Joseph O.P. (2020). Effect of Ethanol Leaf Extract of Pterocarpus santalinus Extract on Kidney of Wister Rats. Page 35-47.

25. Builder M.I., Joseph S.O, Olugbemi T.O. and Akande, T Toxicity. (2020). studies of extract of African Mistletoe: Agelanthus Dodoneifolius Polh and Wiens in Rats. Nigeria biomedical Journal. Page 113-130

26. Builders M. I., Joseph S. O. And B. Peter U. (2020). A Survey of Wound Care Practices by Nurses in a Clinical Setting. International Journal of Healthcare and Medical Sciences. Vol. 6, Issue. 5, Page 74-81.

27. Joseph O. S., Builders M., Joseph O. T. (2020). Effect of Caffeine on Diazepam - Induced Sedation and Hypnosis in Wister Rat. Global Scientific Journal. Vol. 8, Issue 9. Page 451-466