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Subacute toxicity study of aqueous root extract of *Terminalia schimperiana* in male Wistar rats

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ABSTRACT

The effect of administration of aqueous extract of *Terminalia schimperiana* root, "a medicinal plant", on some 'biomarker' enzymes, hematology parameters, liver function and kidney function parameters of rat cellular system was investigated. The aqueous extract was administered orally to male wistar rats (*Rattus norvegicus*) at various doses (1000, 2000, 3000 mg/kg body weight) daily for 21 days and the rats were sacrificed under chloroform anesthesia after 1, 7 and 21 days of oral administration. The administration of the aqueous extract of *Terminalia schimperiana* root for 21 days resulted in significant ($P < 0.05$) increase in packed cell volume and red blood cells level when compared with the control but were all within the normal test range. The differentials remained normal and the white blood cells level remained constant throughout the test period but increased after day 21 of the administration. Aspartate transaminase, alkaline phosphatase and acid phosphatase serum activities significantly ($P < 0.05$) increased, while the serum activities of alanine transaminase and gamma glutamyl transferase significantly ($P < 0.05$) reduced after 21 days of administration when compared with the control but they all fell within the normal test range. The extract produced (out of normal test range) significant ($P < 0.05$) increase in the serum albumin and total bilirubin. The kidney function parameters level was normal for sodium and potassium while the levels of creatinine and urea increased when compared with the control but were within the normal test ranges. The extracts did not have deleterious effect on the male wistar rat organs at the dosages investigated, therefore, studies for extended period is suggested to determine if the prolonged continuous use of the extract might cause challenge on the functional capacity of the organs.

1. Introduction

Terminalia schimperiana root has medicinal potentials which has been used locally for treatment of different conditions. In Africa, the local and urban populace continue to use the infusion of *Terminalia schimperiana* plant (Idi in Yoruba language) for many ethnophamacology purposes. Pulverized root and root bark are applied for treatment of burns, wounds and skin diseases. Root powder is used for epilepsy treatment, root decoctions are used for malaria, hepatitis and cough treatment. The locals also use the roots commonly as chewing sticks for dental care, laxative, tonic and aphrodisiac. Bark decoctions are administered as purgative, used to treat malaria and diarrhea. Leaf decoctions are applied to treat burns, headache, malaria, stomach-ache, hepatitis, amenorrhoea, cough, asthma, diabetes, obesity and elephantiasis while the fruits serve as vermifuge [1]. *Terminalia schimperiana* belongs to the order: myrtales, family: Combretaceae, genus: Terminalia and species: schimperiana. It is a broad-leaved small tree which might reach up to 7–14 m, it has about 9–15 cm long and 3–8 cm broad leaves that are alternate, simple, and elliptic to obovate in shape [2]. It can be found in closed forest as part of the dominant tree species of the forest canopy and can also be found in open forest habitats with more than 1300 mm of rainfall per year [2]. In our previous studies, the aqueous root extract of *Terminalia schimperiana* root was found to have androgenic potentials which may enhance male sexual function and increase reproductive hormone level [3], also the hydroethanol and saponin fractions has been found to have antioxidant activities [4]. However, apart from the medicinal potentials, information on its toxicological implications is not much to the best of our knowledge. Therefore this study was undertaken to provide information on the possible injury that might be caused by the root extract on cells and

Abbreviations: PCV, packed cell volume; RBC, red blood cell; WBC, white blood cell; Cont, control; Neut, neutrophils; Lmph, lymphocytes

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organs of the animal model system by evaluating the activity levels of serum "marker" enzymes, liver function, kidney function and hematology parameters, in male wistar rats. The monitoring of these parameters in the serum will help in comparing the changes in activities with those of the tissues and thus would evaluate its safety.

2. Materials and methods

The roots of *Terminalia schimperiana* were collected from their natural habitat in a local government area of Nigeria while the identification and authentication of plant was carried out at the Department of Botany Ladoke Akintola College of science and technology, Ogbomoso. The plant root was cut into pieces, oven- dried at 40°c and pulverized into powder using an electric grinder. Under constant shaking for 48 h at room temperature, 200 g of the powder was extracted in 2000 ml of distilled water. Whatman no.1 filter paper was used for filtration, then after, the filtrate was lyophilized. The percentage yield was calculated and the resultant yield was reconstituted in distilled water to give the required doses of 1000, 2000 and 3000 mg/kg body weight used for this study. Male wistar rats (*Rattus norvegicus*) weighing between 250–280 g and 4 months old were collected from the animal house and were handled according to the international standard recommendation for animal handling and the use was approved by the University research ethics committee. The assay kits for gamma-glutamyl transferase, alkaline phosphatase, acid phosphatase, bilirubin and urea were products of Human ELISA kit, Gessellscchaftfiir Biochemica und Diagnostica mbh Max-planck-Ring21-D65205 Wiesbaden-Germany. All other reagents used were of analytical grade and were prepared in all glass distilled water.

2.1. Ethical consideration

The rats were placed in a well-ventilated house of 28–31°c temperature, 50–55% humidity, in clean aluminum cages and were maintained on standard mouse pellets (Bendel Feeds and Flour Mills Ltd. Ewu, Nigeria) and tap water. They were handled according to the international standard recommendation for animal care and use. The research was approved by Jagadguru Sri Shivarathreeshwara college of Pharmacy research ethics committee (Reg No.118/PO/ReBi/1999/CPCSEA).

2.2. Experimental design for acute toxicity study

In order to discover the adverse effect of the extract, food was withdrawn over night from male rats, they were treated orally with the limit dose of 2000 mg/kg and higher dose of 4000 mg/kg in accordance with the guidelines of OECD 423. They were observed for14 days for signs of toxicity i.e. food and water intake and any other overt signs of toxicity such as salivation, ptosis, rhinorrhea, squinted eyes, writhing, convulsions, tremors, yellowing of fur, lachrymation and loss of hair. Stress (such as erection of fur) and changes in behavior (such as spontaneous movements in the cage, climbing, cleaning of face, non-genital self-grooming) were also observed.

2.3. Animal grouping and extract administration

For the toxicity studies, 60 male rats were randomly grouped into four groups (A, B, C and D) of 15 animals each. Rats in groups B, C and D were orally administered with 1 ml of the plant extract once daily at 24 h interval at the dosages of 1000, 2000, 3000 mg/kg body weight respectively for 21 days (pharmacologically effective dosages). Group A, which serves as the control, received 1 ml of the vehicle (distilled water) and was treated exactly like the test groups. All administrations were done daily at the same time of between 0900hrs-1000hr. The experimental rats were allowed free access to rat pellets and tap water after the daily dose of the extract/distilled water. The rats were sacrificed after 1, 7 and 21 days of oral administration and toxicity assessment was carried out on blood and serum. In a study of subacute and subchronic toxicity, assessments of *Acridocarpus smeathmannii* (DC.) Guill &Perr.root in Wister rats was done for 28 days and 90 days toxicity at dose of 2000 mg/kg and higher dose of 4000 mg/kg [5].

2.4. White blood cell (WBC) and differential count

The rats were anaesthetized in a jar containing cotton wool saturated with chloroform. Blood sample was quickly collected by jugular incision into separate bottles with anticoagulant i.e. clean and dry EDTA sample bottles. For serum, the blood from the rats was also collected into clean, dry centrifuge tubes. The samples were allowed to stand for 15 min at room temperature for coagulation to take place. Clear serum was then collected using Pasteur pipette after centrifuging at 33.5 x g for 15 min using Uniscope Laboratory Centrifuge (model SM800B, Surgifriend Medicals, Essex, England). The blood collected was analyzed for parameters like WBC, RBC, and differentials (i.e Complete Blood Count) [6] using 5 part differential Analyzer model: SYSMEX 1000 i series.

2.5. Determination of the effect of aqueous extract of Terminalia schimperiana root on enzyme activity

Kinetic colorimetric method for the determination of aspartate transaminase and alanine transaminase activities were done according to the recommendation of the Expert panel of IFCC (International Federation of Clinical Chemistry, 2006) [7]. Kinetic colorimetric method for the determination of gamma glutamyl transferase activity was done according to method of Persijn and van der slik (1976) [8] in which gamma glutamyl transferase catalyzes the transfer of the glutamyl from a peptide to an acceptor molecule, glycylglycine. Optimized standard method according to the recommendations of the German Clinical Chemistry Association was used to determine alkaline phosphatase during which p-nitrophenyl phosphate (PNPP) was hydrolyzed to para-nitrophenol and phosphate at pH 10.1. Acid phosphatase activity was determined by the method of Hillman (1971) [9] in which 1-Naphthylphosphate was hydrolyzed by the enzyme to phosphate and 1-naphtol.

2.6. Determination of liver function

The tests carried out on serum for liver function tests, include; serum protein, bilirubin, globulin and albumin. Serum protein concentration was determined using the Biuret method [10]. Using the method described by Tietz, (1995) [11], serum total bilirubin was determined based on the principle of bilirubin in the serum that is coupled with diazotized sulphanilic acid to form azobilirubin while serum globulin level was determined by subtracting the concentration of serum albumin from the total serum protein content. Serum albumin was determined by the method of Doumas, et al. [12], based on the ability of bromocresol green (BCG), pH 4.2, to bind albumin which results in the formation of a complex that exhibits optical properties different from dye.

2.7. Determination of kidney function

The functional state of the kidney was assessed by measuring the concentration of some metabolites or electrolytes like serum urea, creatinine, potassium and sodium in the serum. The principle is the Humalyte plus system that uses I.S.E. (ion selective electrode) methodology to measure potassium in biological fluids was used. Serum samples were analyzed by automated electrolyte analyzer (Humalyte plus) and the concentration recorded [13,14].

2.8. Statistical analysis

Results were expressed as the mean of five replicates ± SD. Means were analyzed using a one-way ANOVA and values at $P < 0.05$ were considered statistically significant [15].

3. Results and discussion

3.1. Adverse effects of the crude extract

The food and water intake of all the treated Wistar rats remained similar to those of controls and there was no significant depression in body weight. Also, there were neither treatment-related defects nor overt clinical signs of toxicity, stress or changes in appearance and behavior.

3.2. Toxicity of aqueous root extract of Terminalia schimperiana on hematology

The effect of foreign compound like plant extract on the blood components, such as PCV, RBC, WBC, neutrophils, lymphocytes etc. can be used to explain blood related functions of plant extracts [16] and also toxicity of some herbs at the limit dose of 2000 mg/kg and higher dose of 4000 mg/kg, have been assessed and reported [5]. Toxicity of aqueous extract of *Terminalia schimperiana* on the PCV values for 1000 and 2000 mg/kg dosage group for day 1, and 1000 mg/kg dosage for days 7 and 21 were not significantly different from control throughout the period of administration. PCV values at the dose of 3000 mg/kg bodyweight on day 1increased with values of 37 ± 5.7%, PCV at 2000 and 3000 mg/kg dosages on day 7 increased with 38 ± 2.1% and 38 ± 5.6% respectively, while PCV at 2000 and 3000 mg/kg dosages on day 21 increased with 41 ± 3.9% and 42 ± 3.2% respectively when compared with the control value of 35 ± 1.1%. However, the increased values were still within the normal PCV test range of 33–50% for rats. The RBC level when compared with the control value of 6.91 ± 2.1 ($x10^{-6}$/μL), showed increased values of 8.69 ± 1.9 ($x10^{-6}$/μL) and 8.71 ± 2.0 ($x10^{-6}$/μL) for 2000 and 3000 mg/kg dosages respectively on day 1, 9.02 ± 3.1 ($x10^{-6}$/μL) and 10.11 ± 2.1 ($x10^{-6}$/μL) for 2000 and 3000 mg/kg dosages respectively on day 7, and 9.73 ± 2.7($x10^{-6}$/μL) and 9.58 ± 3.1($x10^{-6}$/μL) for 2000 and 3000 mg/kg dosages respectively on day 21 but all the RBC values fell within the normal range of 6.56–9.75 ($x10^{-6}$/μL) (Table 1). The increase in the RBC and PCV might be due to increase in the rate of production of erythrocytes, it may also show that that the extract exhibited erythropoietin potential and this might be that the oxygen-carrying capacity of the blood and amount of oxygen delivered to the tissues was enhanced by the extract administration. In a study, aqueous leaves extract of *Ocimum gratissimum* was reported to have increased the levels of RBC and PCV in rats [17]. Administration of aqueous extract of *Terminalia schimperiana* root for seven days produced no significant difference ($P > 0.05$) WBC (normal range: 5.5–12.6/μL), lymphocytes (normal range: 3.78–8.24/ μL) and neutrophils (normal range: 0.30-0.71/μL) when compared with their controls. Further administration for 21 days resulted in no significant increase in neutrophils and lymphocytes but WBC showed a significant increase by the end of 21st day daily doses at the 3000 mg/kg body weight dosage with the value of 14.77 ± 1.6 ($x10^{-6}$/μL) when compared with the control value of 12.66 ± 3.8^{-6}/μL and the increase was out of the normal range. The initial normal levels of WBC, lymphocytes and neutrophils at all the dosages investigated is an indication that the extract did not cause any adverse challenge on the immune system while the out of normal range increase of WBC after 21 days of administration might indicate a challenge on the immune system by the plant extract probably as a result of prolonged period of continuous use. These hematology findings are further supported by other toxicity studies by Awotunde and Yakubu [18] on *Massularia acuminata* stem.

Table 1
Effect of administration of aqueous extract of *Terminalia schimperiana* root on some hematological indices of male wistar rats.

Indices	Doses Day 1 Cont	1000 mg/kg	2000 mg/kg	3000 mg/kg	Doses Day 7 Cont	1000 mg/kg	2000 mg/kg	3000 mg/kg	Doses Day 21 Cont	1000 mg/kg	2000 mg/kg	3000 mg/kg
PCV (%)	35 ± 1.1[a]	35 ± 2.3[a]	35 ± 4.2 [a]	37 ± 5.7 [b]	35 ± 1.1[a]	35 ± 5.1[a]	38 ± 2.1 [b]	38 ± 5.6 [b]	35 ± 1.1[a]	35 ± 6.3 [a]	41 ± 3.9 [b]	42 ± 3.2 [b]
RBC ($x10^{-6}$/μL)	6.91 ± 2.1[a]	6.87 ± 2.8 [a]	8.69 ± 1.9 [b]	8.71 ± 2.0 [b]	6.91 ± 2.1 [a]	6.72 ± 1.8 [a]	9.02 ± 3.1 [b]	10.11 ± 2.1 [c]	6.91 ± 2.1[a]	6.76 ± 2.9[a]	9.73 ± 2.7 [b]	9.58 ± 3.1 [b]
WBC ($x10^{-6}$/μL)	12.66 ± 3.8 [a]	11.89 ± 2.2 [a]	11.75 ± 4.1 [a]	11.89 ± 3.1 [a]	12.66 ± 3.8 [a]	12.30 ± 2.9 [a]	12.32 ± 2.0 [a]	12.33 ± 2.2 [a]	12.66 ± 3.8 [a]	11.26 ± 0.9 [a]	13.00 ± 2.0 [a]	14.77 ± 1.6 [b]
Neut($x10^{-3}$/μL)	0.68 ± 0.9 [a]	0.63 ± 0.21 [a]	0.62 ± 0.22 [a]	0.62 ± 0.32 [a]	0.66 ± 0.12 [a]	0.64 ± 0.31[a]	0.66 ± 0.23 [a]	0.67 ± 0.32 [a]	0.68 ± 0.9 [a]	0.62 ± 0.31 [a]	0.61 ± 0.22 [a]	0.62 ± 0.32 [a]
Lymph($x10^{-3}$/μL)	3.04 ± 1.4 [a]	3.29 ± 0.8 [a]	4.10 ± 1.2 [a]	4.00 ± 2.2 [a]	3.04 ± 1.4 [a]	2.62 ± 1.8 [a]	2.66 ± 2.0 [a]	2.58 ± 0.9 [a]	3.04 ± 1.4 [a]	3.42 ± 1.8 [a]	3.22 ± 2.4[a]	3.20 ± 2.0 [a]

Values are meana ± SD (n = 5); superscripts b and c, different from the control a, for each day and for each parameter are significantly different ($P < 0.05$)(Table Key: PCV – Packed Cell Volume, RBC – Red Blood Cell, WBC – White Blood Cell, Cont – Control, Neut – Neutrophils, Lmph – Lymphocy.

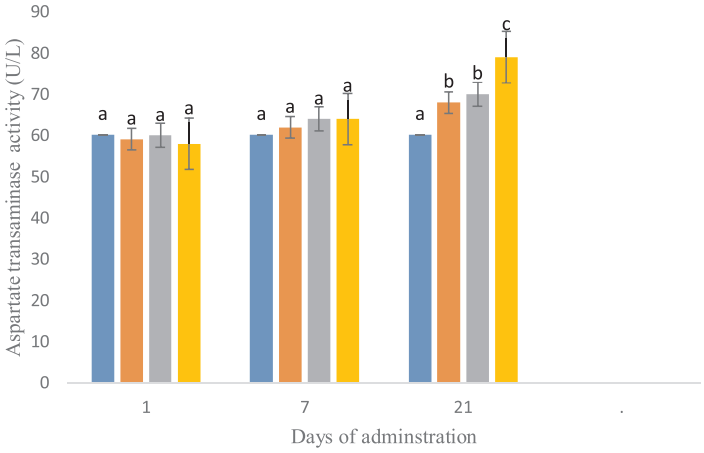

Fig. 1. Effect of administration of aqueous extract of *Terminalia schimperiana* root on male rat serum Aspartate transaminase activity. Group A (control) was orally administered with 1 ml of the vehicle, groups B, C and D were orally administered with 1 ml of the extract (1000, 2000, 3000 mg/kg body weight) respectively once daily at 24 h interval for 21 days. Results obtained are expressed as the mean ± SD (n = 5). $^{a–c}$ Bars carrying superscripts different from the control for each day are significantly different ($P < 0.05$), one way ANOVA.

3.3. Toxicity of the aqueous root extract on enzyme activity

The measurement of the activities of 'marker' enzymes in tissues and body fluids can be used in assessing the degree of assault and the toxicity of a chemical compound on organ/tissues [19,20]. In pursuance of this aim the levels of liver enzymes in serum were determined as described in the methods. The patterns of liver-function enzymes are shown in Figs. 1–5.As it can be observed in Fig. 1, aspartate transaminase activity increased ($P > 0.05$) following the repeated oral administration of aqueous extract of *Terminalia schimperiana* root on day 21 when compared with the control with the values of 68, 70 and 79 U/L but the increased values fell within the normal range of 45.7–80.8 U/L. Aspartate transaminase a marker enzyme for liver damage is widely distributed throughout the body and found primarily in the heart, liver, skeletal muscle, and kidney. Liver disease is the most important cause of increased transaminase activity in the serum. The normal activity of serum aspartate transaminase following the repeated oral administration of aqueous extract of *Terminalia schimperiana* root for 7 days despite the increase in activity on the day 21 when compared with the control, could be that the extract did not affect the integrity of the liver cell but increased the level on the day 21 of the administration probably as a result of prolonged continues use. Similarly, the alanine transaminase activity was not significantly different ($P > 0.05$) from the control at days 1 and 7 period but decreased with 30 U/L, 28 U/L and 27 U/L for 1000, 2000 and 3000 mg/kg respectively when compared with the control's enzyme activity of 35 U/L on day 21 following the administration of crude aqueous extract of *Terminalia schimperiana* root (Fig. 2) but still fell within the normal range of 17.5–30.2 U/L. Alanine transaminase, a marker enzyme for liver damage could be found primarily in the liver and kidney and it is exclusively cytoplasmic. While the normal level indicate normal functioning liver, the decrease on day 21 following the administration of aqueous extract of *Terminalia schimperiana* root compared with the control could be that the extract suppressed the levels of ALT. In another study of hepatotoxicity in rats, it was also reported that pretreatment with ethanolic extract of Lepidium sativum and silymarin significantly reduced the level of serum alanine transaminase [21]. The effect of administration of aqueous extract of *Terminalia schimperiana* root on Gamma glutamyl transaminase (normal range: 1–5.3 U/L) in male rat is shown in Fig. 3, compared with the control value of 3.5 U/L, extract administration at all the doses produced no significant difference ($P > 0.05$) in the enzyme activity on day 1 but administration for 7 days resulted in significant decrease in the enzyme activity ($P > 0.05$) with the values of 1.25 U/L, 2.25 U/L, 2.75 U/L for 1000, 2000 and 3000 mg/kg respectively, while further administration for 21 days resulted in significant decrease in enzyme activity ($P > 0.05$) of the liver with 2.25 U/L, 2.5 U/Land 3.1 U/L for 1000, 2000 and 3000 mg/kg respectively. GGT is a microsomal enzyme present in hepatocytes and biliary epithelial cells, renal tubules, pancreas and intestine [22]. Serum GGT activity mainly attributed to hepatobiliary system even though it is found in more concentration in renal tissue [23]. It is also important in glutathione metabolism and reabsorption of amino acids from the glomerular filtrate and intestinal lumen. The decrease in serum GGT activity (Fig. 3) might be attributed to liver challenge which may be as a result of prolonged continuous use of the extract. If the alterations of GGT activity caused by the extract continues beyond the normal test range, it may adversely affect the metabolism of glutathione and reabsorption of amino acids from the glomerular filtrate and intestinal lumen. The effect of administration of aqueous extract of *Terminalia schimperiana* root on the acid phosphatase of male rat serum is shown in Fig. 4. Extract administration resulted in significant increase in serum acid phosphatase activity throughout the experimental period with day 1 enzyme activity of 12.2 U/L, 14.1 U/L,

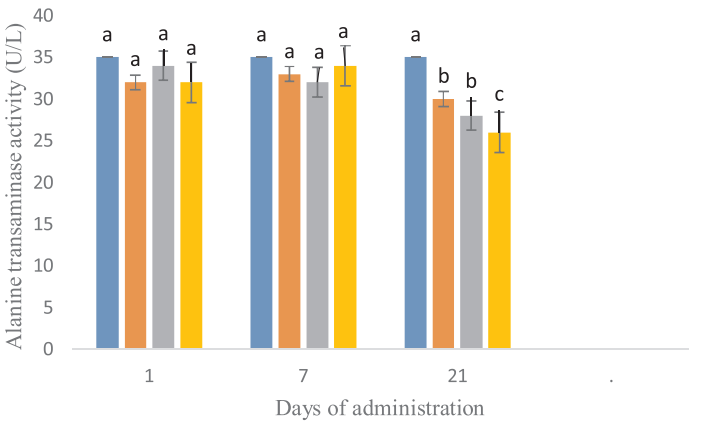

Fig. 2. Effect of administration of aqueous extract of *Terminalia schimperiana* root on male rat serum Alanine transaminase activity. Group A (control) was orally administered with 1 ml of the vehicle, groups B, C and D were orally administered with 1 ml of the extract (1000, 2000, 3000 mg/kg body weight) respectively once daily at 24 h interval for 21 days. Results obtained are expressed as the mean ± SD (n = 5). $^{a–c}$ Bars carrying superscripts different from the control for each day are significantly different ($P < 0.05$), one way ANOVA.

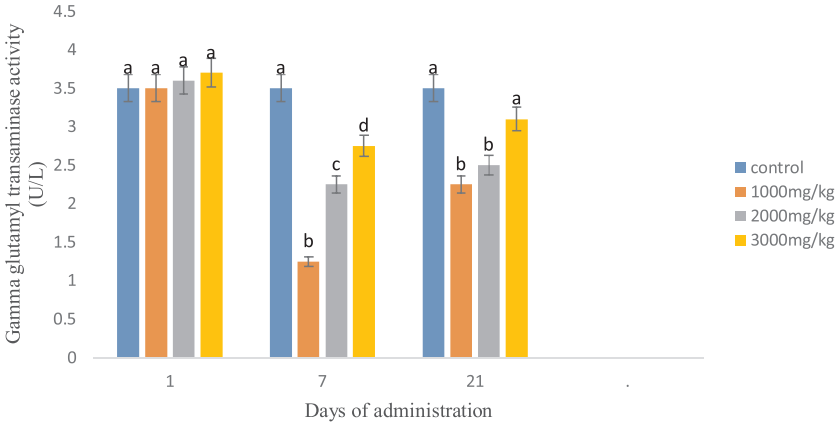

Fig. 3. Effect of administration of aqueous extract of *Terminalia schimperiana* root on male rat serum Gamma Glutamyl transaminase activity. Group A (control) was orally administered with 1 ml of the vehicle, groups B, C and D were orally administered with 1 ml of the extract (1000, 2000, 3000 mg/kg body weight) respectively once daily at 24 h interval for 21 days. Results obtained are expressed as the mean ± SD (n = 5). [a–d] Bars carrying superscripts different from the control for each day are significantly different ($P < 0.05$), one. way ANOVA.

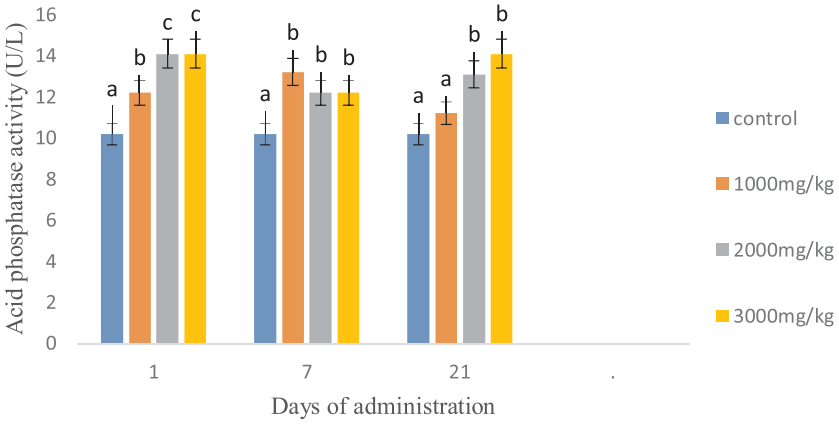

Fig. 4. Effect of administration of aqueous extract of *Terminalia schimperiana* root on male rat serum acid phosphatase activity. Group A (control) was orally administered with 1 ml of the vehicle, groups B, C and D were orally administered with 1 ml of the extract (1000, 2000, 3000 mg/kg body weight) respectively once daily at 24 h interval for 21 days. Results obtained are expressed as the mean ± SD (n = 5). [a–c] Bars carrying superscripts different from the control for each day are significantly different ($P < 0.05$), one way ANOVA.

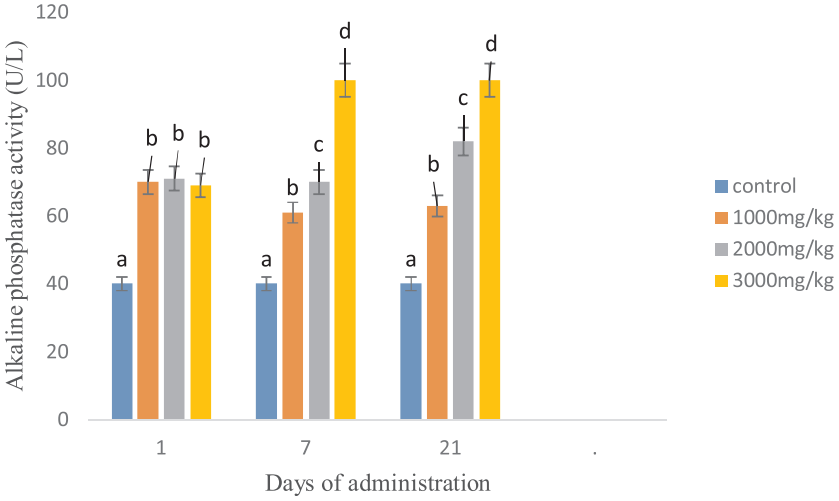

Fig. 5. Effect of administration of aqueous extract of *Terminalia schimperiana* root on male rat serum alkaline phosphatase activity. Group A (control) was orally administered with 1 ml of the vehicle, groups B, C and D were orally administered with 1 ml of the extract (1000, 2000, 3000 mg/kg body weight) respectively once daily at 24 h interval for 21 days. Results obtained are expressed as the mean ± SD (n = 5). [a–d] Bars carrying superscripts different from the control for each day are significantly different ($P < 0.05$), one way ANOVA.

14.1 U/L for 1000, 2000 and 3000 mg/kg respectively, day 7 enzyme activity of 13.2 U/L, 12.2 U/L and 12.2 U/L for 1000, 2000 and 3000 mg/kg respectively and day 21 activity of 11.2 U/L,13.1 U/L and 14.1 U/L for 1000, 2000 and 3000 mg/kg respectively when compared with the control's enzyme activity of 10.2 U/L. However, the increases fell within the normal activity range of 9–16 U/L. Acid phosphatase is a 'marker' enzyme for the lysosomal membrane and elevated serum acid phosphatase activity (Fig. 4) could be due to the effect of the component of the extract leading to *denovo* synthesis of the enzyme molecule or *insitu* contribution or loss of protein from the tissue of other organ. This trend of increase was also extended to the serum alkaline phosphatase activity of the male rats following the administration of the plant extract at all the doses of 1000, 2000 and 3000 mg/kg body weight investigated (Fig. 5), but the increases were within the test range of 56.8-128U/L. Alkaline phosphatase (ALP) is a 'marker' enzyme for the plasma membrane and endoplasmic reticulum [24]. It is often used to evaluate plasma membrane integrity. Any alteration in the tissue enzyme level and serum enzyme level might likely indicate damages to the external boundary of cells (plasma membrane) [25]. The significant increase in the serum alkaline phosphatase activity of the male rat (Fig. 5) may be attributed to induction in the enzyme synthesis probably by *denovo* which if the increase exceeds the normal test range might cause indiscriminate hydrolysis of phosphate ester of the tissues and such increase in alkaline phosphatase activities can be life

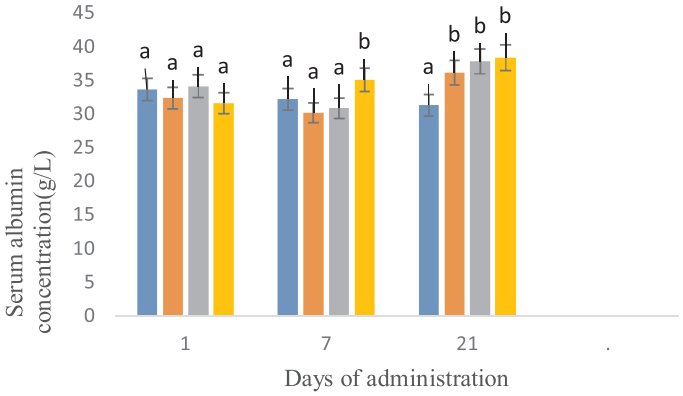

Fig. 6. Effect of administration of aqueous extract of *Terminalia schimperiana* root on male rat serum albumin concentration. Group A (control) was orally administered with 1 ml of the vehicle, groups B, C and D were orally administered with 1 ml of the extract (1000, 2000, 3000 mg/kg body weight) respectively once daily at 24 h interval for 21 days. Results obtained are expressed as the mean ± SD (n = 5). [a–b] Bars carrying superscripts different from the control for each day are significantly different ($P < 0.05$), one way ANOVA.

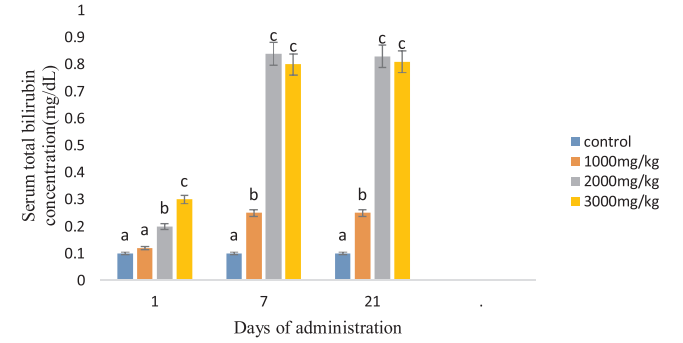

Fig. 7. Effect of administration of aqueous extract of *Terminalia schimperiana* root on male rat serum total bilirubin concentration. Group A (control) was orally administered with 1 ml of the vehicle, groups B, C and D were orally administered with 1 ml of the extract (1000, 2000, 3000 mg/kg body weight) respectively once daily at 24 h interval for 21 days. Results obtained are expressed as the mean ± SD (n = 5). [a–c] Bars carrying superscripts different from the control for each day are significantly different ($P < 0.05$), one way ANOVA.

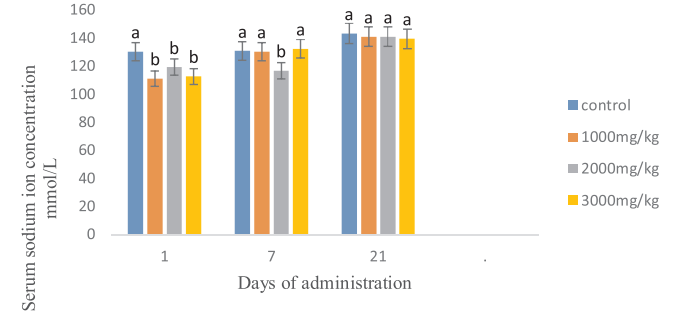

Fig. 8. Effect of administration of aqueous extract of *Terminalia schimperiana* root on male rat serum sodium ion concentration. Group A (control) was orally administered with 1 ml of the vehicle, groups B, C and D were orally administered with 1 ml of the extract (1000, 2000, 3000 mg/kg body weight) respectively once daily at 24 h interval for 21 days. Results obtained are expressed as the mean ± SD (n = 5). [a–b] Bars carrying superscripts different from the control for each day are significantly different ($P < 0.05$), one way ANOVA.

threatening to cells. Therefore from the enzyme study above, the extract caused induction of some enzyme activities and suppression of some others when compared with the control but the changes were all within their normal test ranges hence, the extract did not have adverse effect on the rat liver enzyme activities. This study further supports the histology finding of Awotunde et al., [26] on histopathologic examination of the liver, kidney and testes which revealed mild changes in the architecture of the tissues after the administration of aqueous extract of *Terminalia schimperiana* root to male wistar rats.

3.4. Toxicity of the aqueous root extract on liver function parameters

The liver function indices evaluated in this study are useful parameters used to indicate impairment in the functional capacity of the liver. Serum albumin concentration (Fig. 6) did not show any significant difference ($P > 0.05$) when compared with the control on days 1 and 7, but it increased on day 21 with dose dependent values of 36.08, 37.75 and 38.33 g/L for 1000, 2000 and 3000 mg/kg respectively when compared with the control value of 31.25 g/L. However, the increase was out of the albumin normal test range of 31–35 g/L. The increase in the serum albumin at all the doses after 7 days of administration (Fig. 6) might be due to the increased rate of hepatic synthesis of albumin as a result of the extract administration, it might also be that the extract caused dehydration in the rat or it might be as a result of high dietary protein intake. Bilirubin a yellow breakdown product of normal heme catabolism is excreted in bile, and its levels are elevated in certain diseases, it is an index of liver damage and the major breakdown product of red blood cells. In the liver it is conjugated with glucuronic acid, making it soluble in water [27]. The serum total bilirubin concentration (normal range: 0.1-0.55 mg/dL) was increased throughout the experimental period following the administration of the aqueous extract of *Terminalia schimperiana* root (Fig. 7) with concentrations of; 0.12, 0.2 and 0.3 mg/dL at 1000, 2000 and 3000 mg/kg respectively for day1, 0.25, 0.84 and 0.80 mg/dL at 1000, 2000 and 3000 mg/kg respectively for day7 and 0.25,0.83 and 0.81 mg/dL at 1000, 2000 and 3000 mg/kg respectively for day 21 when compared with the control concentration of 0.1 mg/dL. The increased serum total bilirubin (Fig. 7) might be because more aged red blood cells are being destroyed leading to more production of bilirubin, blockage of the bile duct, buildup of direct bilirubin and escape from the liver, ending up in the blood thus, this might affect the ability of the liver to transform bilirubin to the bile pigment-bilirubin glucuronide. These Results of enzyme activities and liver functions are similar to the findings reported by Tano et al. [16] on toxicity of methanol extract of *Terminalia Schimperiana.* In another study on the role of *Nigella sativa Linn* oil against subacute tramadol-induced hepatotoxicty, nephrotoxicity and oxidative stress in male albino rats, *Nigella sativa Linn* oil elevated the level of bilirubin and urea [28].

3.5. Toxicity of the aqueous root extract on kidney function indices

The renal function can be checked through the monitoring of secretory products electrolyte concentration of sodium, potassium, urea and creatinine. These electrolytes are usually required to assess the normal functioning capacity of different parts of the functioning unit of the kidney [29]. Electrolytes occur in large amounts in both extracellular and intracellular fluids and due to their ability to dissociate readily into their constituent ions, they are very important in the transfer of water and electrolytes between the extracellular and intracellular compartments [30].The effects of administration of aqueous

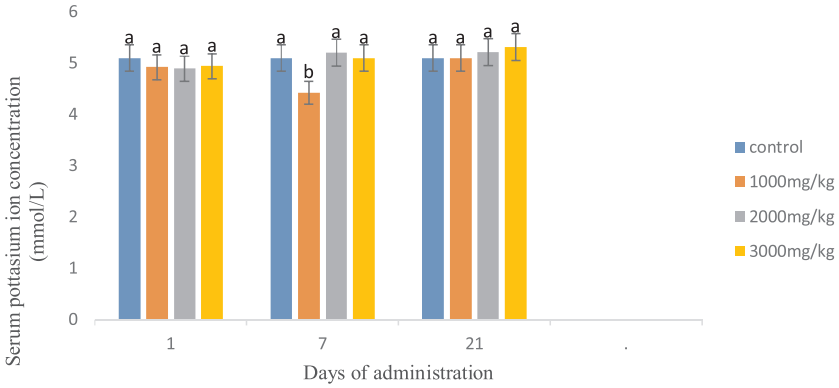

Fig. 9. Effect of administration of aqueous extract of *Terminalia schimperiana* on male rat serum potassium ion concentration. Group A (control) was orally administered with 1 ml of the vehicle, groups B, C and D were orally administered with 1 ml of the extract (1000, 2000, 3000 mg/kg body weight) respectively once daily at 24 h interval for 21 days. Results obtained are expressed as the mean ± SD (n = 5). [a–b] Bars carrying superscripts different from the control for each day are significantly different ($P < 0.05$), one way ANOVA.

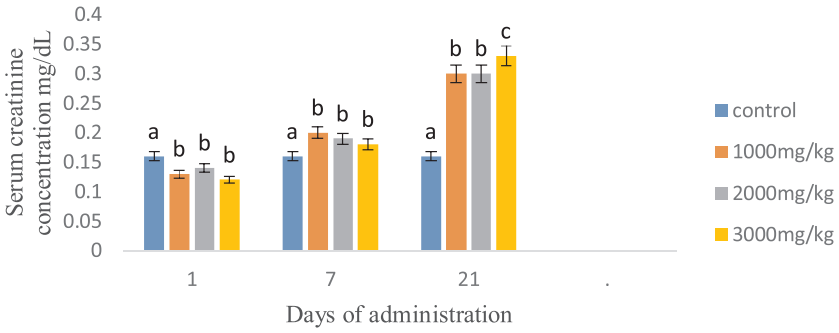

Fig. 10. Effect of administration of aqueous extract of *Terminalia schimperiana* root on male rat serum creatinine concentration. Group A (control) was orally administered with 1 ml of the vehicle, groups B, C and D were orally administered with 1 ml of the extract (1000, 2000, 3000 mg/kg body weight) respectively once daily at 24 h interval for 21 days. Results obtained are expressed as the mean ± SD (n = 5). [a–b] Bars carrying superscripts different from the control for each day are significantly different ($P < 0.05$), one way ANOVA.

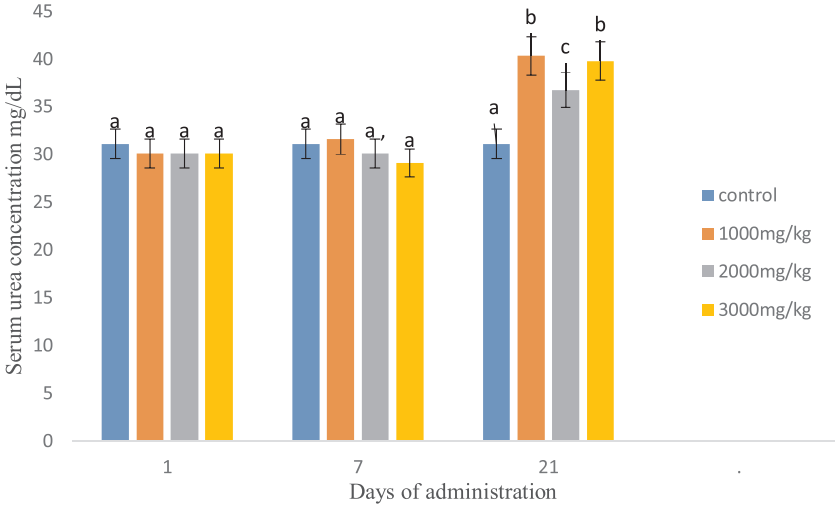

Fig. 11. Effect of administration of aqueous extract of *Terminalia schimperiana* on male rat serum urea concentration. Group A (control) was orally administered with 1 ml of the vehicle, groups B, C and D were orally administered with 1 ml of the extract (1000, 2000, 3000 mg/kg body weight) respectively once daily at 24 h interval for 21 days. Results obtained are expressed as the mean ± SD (n = 5). [a–c] Bars carrying superscripts different from the control for each day are significantly different ($P < 0.05$), one way ANOVA.

extract of *Terminalia schimperiana* root on some indices of kidney function of male rats are shown in Figs. 3–6. Similar to Tano et al., [16] reports on renal function parameters, extract administration at all the doses resulted in no significant difference ($P < 0.05$) in sodium ion (normal range: 110–150 mmol/L) and potassium ion (normal range: 4.3–5.8 mmol/L) concentration after day 21 of the administration when compared with the control (Figs. 8 and 9).The constancy in sodium ions at various doses following the administration of the extract after 21 days of the administration (Fig. 8) may be that the administration of the extract did not result in loss of Na^+ from body fluids. It may also be attributed to normal production of aldosterone and other mineralocorticoids which in turn produced normal reabsorption of Na^+ [31]. The constancy in potassium ions (Fig. 9) following the administration of the extract indicates no possible adverse effect on the sodium/potassium pump that maintains the constancy of the extracellular concentration of potassium. Such normal pattern suggests that there is no tubular nor glomerular dysfunction. In contrast, the serum concentration of creatinine and urea were both increased at all treatment doses on day 21. The concentrations of creatinine increased to 0.3, 0.3 and 0.33 mg/dL for 1000, 2000 and 3000 mg/kg respectively when compared with the control's concentration of 0.16 mg/dL but are all within the normal creatinine test range of 0.1-0.8 mg/dL. Measurement of serum creatinine is a simple test commonly used as indicator of renal function. Constancy of endogenous creatinine production and its release into the body fluids at a constant rate, and constancy of plasma levels of creatinine over 24 h of a day, makes creatinine a useful endogenous substance. Increase in serum creatinine content (Fig. 10) observed with the extract on day 21 of administration might be attributed to diet composition of the rat feed or the effect of extract on the nephron. The concentration of urea with the normal urea range of 30–41 mg/dL, increased to 40.3, 36.75 and 39.78 mg/dL for 1000, 2000 and 3000 mg/kg respectively when compared with the control's concentration of 31.1 mg/dL (Figs. 10 and 11). Urea is the metabolic product of protein catabolism and increase in serum urea might hamper

the kidney function if it is not controlled accordingly. The increase in serum urea (Fig. 11) at all doses on day 21 of administration in this study might imply enhanced production and excretion of urea maybe as a result of the diet composition of the rat feed. In a study of sub-acute administration of nicotine at lower doses of 1, 2 and 4 mg/kg i.p. for 28 days, there was beneficial alterations in plasma and urine level of creatinine, urea and uric acid ($p < 0.05$) as well as plasma and urine electrolyte level (Na^+ and K^+) in both sexes ($p < 0.05$). There was also a significant improvement in creatinine clearance ($p < 0.05$) of renal function in wistar rats [32].

4. Conclusion

From this study, the extract caused induction of some enzyme activities and suppression of some others when compared with the control but the changes were all within their normal test ranges, hence, the extract might not have adverse effect on the rat liver enzyme activities. The normal range levels in the activity of the marker enzymes after repeated administration of the aqueous extract for 21 days suggests that there is no damage inflicted on the plasma membranes which might compromise their integrity. The RBC and PCV levels increased and this might be because of increase in the rate of production of erythrocytes which suggests that that that the extract might have exhibited erythropoeitin potential and this might enhance the oxygen-carrying capacity of the blood and amount of oxygen delivered to the tissue. The normal levels of WBC and differentials at all dose levels might be an indication that there is no challenge on the immune system, while further change might indicate challenge on the immune system by the plant extract. Normal level of sodium ion suggests normal production of aldosterone and other mineralocorticoids which in turn produced normal reabsorption of Na^+, while the constancy in potassium ions might also indicate no possible adverse effect on the sodium/potassium pump that maintains the constancy of the extracellular concentration of potassium. However, the increase in urea and creatinine might challenge the functional capacity of the organs. The results of this study thus indicate that acute administration of *Terminalia schimperiana* did not have significant adverse effect on the biochemical indices evaluated but the prolonged use might lead to labialization of the cell plasma membrane and cause disruption of the ordered lipid bilayer of the plasma membrane thus resulting in leakage of the enzymes to the extracellular fluid.

Declaration of Competing Interest

The authors declare that we have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] R.H.M.J.A. Lemmens, G.H. Schmelzer, Gurib-Fakim (Eds.), *Terminalia schimperiana* Hochst. Plant Resources of Tropical Africa, PROTA Foundation, Wageningen, NChloroformlands, 201311(2).
[2] E.W. Jones, The Cece Forest Reserve, Northern Nigeria, J. Ecol. 51 (2) (1993) 461–466.
[3] O.S. Awotunde, S.O. Adewoye, Hawumba, Sexual behavior effect of orally administered crude aqueous extract of Terminalia schimperiana root in male Wistar rat, J. Med. Plant Stud. 4 (6) (2016) 243–247 (Personal communication).
[4] O.S. Awotunde, S.P. Dhanabal, Raman Rajeshkumar, M.V.N.L. Chasitainya, Antioxidant activities of Hydro-ethanol and saponin extracts of Terminalia schimperiana root, Der Pharmacia Lettre 9 (11) (2017) (Personal communication).
[5] O.E. Kale, O. Awodele, A.J. Akindele, Subacute and subchronic oral toxicity assessments of Acridocarpus smeathmannii (DC.) guill. & perr. root in Wistar rats, Toxicol. Rep. (6) (2019) 161–175.
[6] Cheesbrough, McArthur, Laboratory Manual for Rural Tropical Hospitals. A Basis for Training Courses, (1976).
[7] IFCC (International Federation of Clinical Chemistry), Kinetic Colorimetric Method for the Determination of ASAT, (2006).
[8] Persijn, W. Van der Slik, A new method for the determiniation of gamma-lglutamyl transferase in serum, J. Clin. Chem. Biochem. (9) (1976) 421–427.
[9] G. Hillman, Z.Klin Chem, Klin. Biochem. 9 (273) (1971).
[10] D.T. Plummer, An Introduction to Practical Biochemistry, 2nd edition, McGraw-Hill, London, 1978, pp. 144–145.
[11] N.W. Tietz, Clinical Guide to Laboratory Tests, 3rd edition, W.B. Saunders Compnay, Philadelphia, 1995.
[12] B.T. Doumas, W.A. Watson, H.G. biggs, Albumin standards and measurement of serum albumin with bromocresol green, Clin. Chem. Act 31 (1971) 87–92.
[13] M.P. Veniamin, C. Varkirtzi, Chemical basis of the carbamidodi-acetyl micro-method for estimation of urea, cirrulline and carbamyl derivatives, Clin. Chem. 16 (1970) 3–6.
[14] K.G. Blass, R.J. Thiebert, L.K. Lam, Study of mechanism of JAFE reactions, Clin. Chem. 12 (7) (1979) 336–343.
[15] B.K. Mahajan, Methods in biostatistics for medical and research workers, in: B.K. Mahajan (Ed.), JAYPEE, Mahajan B.K Brothers Medical Publishers, New Delhi, 1997, pp. 130–155.
[16] K.D. Tano, J.H. Kouadio, W. Yavo, A.J. Djaman, E.I.H. Menan, Toxic effect study of methanol extract of Terminalia glaucesens leaves following single or short-term repetitive oral administration in Swiss mice, Am. J. Biosci. 1 (4) (2013) 85–90.
[17] O.E. Ofem, E.J. Ani, A.E. Eno, Effect of aqueous leaves extract of *Ocimum gratissimum* on hematological parameters in rat, Int. J. Appl. Basic Med. Res. 2 (1) (2012) 38–42.
[18] O.S. Awotunde, M.T. Yakubu, Assessment of enzyme activities and hematological parameters in male wister rats administered with aqueous extract of Massularia acuminata root, Drug Discovery From Herbs, NAM S&T Centre book, 2017 (Personal communication), ISBN: 978-93-86071-46-0.
[19] S.O. Malomo, Toxicological implication of ceftriaxone administration in rats, Nig. J. Biochem. Mol. Biol. 15 (1) (2000) 33–38.
[20] M.T. Yakubu, L.S. Bilbis, M. Lawal, M.A. Akanji, Effect of repeated administration of sildenafil citrate on selected enzyme activities of liver and kidney of male albino rats, Nig. J. Pure Appl. Sci. 18 (2003) 1395–1400.
[21] A.K. A-Asmari, M.T. Athar, H.M. Al-Shahrani, S.I. Al-Dakheel, M.A. Al-Ghamdi, Efficacy of Lepidium sativum against carbon tetra chloride induced hepatotoxicity and determination of its bioactive compounds by GC/MS, Toxicol. Rep. 2 (2015) 1319–1326.
[22] Shivaraj Gowda, Prakash B. Desai, Vinayak V. Hull, Avinash A.K. Math, Sonal N. Vernekar, Shruthi S. Kulkarni, A review on laboratory liver function tests, Pan Afr. Med. J. 3 (2009) 17.
[23] P. Mauro, B. Renze, W. Wouter, A.B. Carl, R. Edward, E.B. David (Eds.), Tietz Text Book of Clinical Chemistry and Molecular Diagnostics. 4th edition, Elsevier, 2006, pp. 604–616.
[24] P.D. Mayne, Clinical Chemistry in Diagnosis and Treatment, 6th edition, Arnold Internaitonal Students' Edition, 1998, pp. 196–205.
[25] M. Sabitha, K.E. Mallika, C.S. Shyamala-Devi, Effect of *Solanum trilobatum* against carbon tetrachloride induced hepatic damage in albino rats, Indian J. Med. 120 (2004) 194–198.
[26] O.S. Awotunde, Adewoye, S.O. Hawumba, J. Joseph, J. Lordrick Alinaitwe, Histopathological and organ-body weight ratio alterations induced by oral administration of aqueous extract of *Terminalia schimperiana* root in some selected organs in male Wistar rats, Int. J. Adv. Res. Sci. Eng. Technol. 4 (2) (2017).
[27] D.E. Baranano, M. Rao, C.D. Ferris, S.H. Snyder, Biliverdinreductase: a major physiologic cytoprotectant, Proc. Natl. Acad. Sci. U. S. A. 99 (25) (2002) 16093–16098.
[28] A. Elkhateeb, I. El Khishin, O. Megahed, F. Mazen, Effect of Nigella sativa Linn oil on tramado;l- induced hepato- and nephrotoxicity in adult male albino rats, Toxicol. Rep. 2 (2015) 512–519.
[29] N.C. Panda, G.P. Talwer, L.M. Stivastava, K.D. Moudgil (Eds.), Textbook of Biochemistry and Human Biology, 2nd edition, Prentice-Hall of India. Private Ltd, 1989, pp. 276–292.
[30] J.F. Zilva, P.R. Panmall, P.D. Mayne, Clinical Chemistry in Diagnosis and Treatment, 5th edition, England Clays Ltd. St Ives Plc, 1991.
[31] N.W. Tietz, E.L. Pruden, O. Siggaard-Anderson, C.A. Burtis, E.R. Ashwood (Eds.), Tietz Textbook of Clinical Chemistry, W. B. Saunders Company, London, 1994, pp. 135–137.
[32] Ojo Rufus Akomolafe, Christian Eseigbe Imafidon, Olaoluwa SesanOlukiran, Ayowole Abraham Oladele, Babatunde OludareAkanji, Sub-acute administration of lower doses of nicotine caused sex-dependent improvement of renal function in Wistar rats, Toxicol. Rep. (4) (2017) 535–542.