



(RESEARCH ARTICLE)



Hepatotoxicity effects of Methanol extract of *Gmelina arborea* leaves on liver enzymes marker in Wistar Rats

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Abstract

Liver diseases are a major cause of mortality and morbidity in developing countries. This study aims at determining the hepatotoxicity effects of methanolic-extracts of *Gmelina arborea* leaves at different concentrations on liver marker enzymes in wistar rats. The experiment was laid out in a complete randomized design (CRD). Fresh leaves of *Gmelina arborea* from the school environments was collected, dried, grinded and extracted using muslin cloth and methanol. The extract was then placed in the oven at about 38°C for dryness. The extract was administered to five groups of wistar rats orally and twice daily for 21 days at doses of 1000mg/kg, 500mg/kg, 250mg/kg, 125mg/kg and group one control group (wasn't administered this extract). At the end of every week (7 days) the blood samples were collected through ocular puncture, and biochemical analysis of Serum test (Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), Total bilirubin (TB) and Conjugated bilirubin (CB) were estimated. There was no mortality observed as a result of administration of this leave extract. Methanol extract of *Gmelina arborea* did not produce significant differences in the body weight, organ weights and feeding habits between control and treated animals. Pathologically, neither gross abnormalities nor histopathological changes were observed. Methanol extract of *Gmelina arborea* was found safe in acute and repeated dose toxicity study when tested in wistar rat.

Keywords: Mortality; Hepatotoxicity; *Gmelina arborea*; Histopathology; Concentrations.

1. Introduction

Medicinal plants are plants which contain substances that could be used for therapeutic purposes or which are precursors for the synthesis of useful drugs (Khare *et al.*, 2004). A plant becomes a medicinal plant only when its biological activity has been ethno-botanically reported or scientifically established. It has been reported that the bases of many modern pharmaceuticals used today for the treatment of various ailments are plants and plant based product and about 80% of world population depends on plants based medicine for their health care (Pullaiah, 2006). Medicinal plants are divine gifts to us from Mother Nature who has kept those remedies in her plant kingdom for mankind to use to fight death from diseases and cure themselves from ailments. It is up to us to seek, to explore, search and reap the benefits of these treasures (Pandey, 2000).

Gmelina arborea is a deciduous tree locally known as *Ghamhar*. *arborea* belongs to family verbenaceae. Its immemorial because it has huge medicinal properties *arborea* is known to different name in different languages like Gomari in Assamese, Gamari, Gambar, Gumbar in Bengali, Shewan, Sivan in Gujarati, Gamhar, Khamara, Khumbhari, Sewan in Hindi, Kulimavu,

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As the largest internal and major organ in the body whose functions include metabolizing, detoxifying and regeneration of cells, the liver secretes the enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP). The levels of these enzymes in the plasma have over the years been used as indicators of liver toxicity. It is established that elevations in AST and ALT (amino-transferases) values indicate early diagnosis of hepatotoxicity and tissue damage (Kamal and Hessah, 2015). Hence, elevations in the levels of these liver enzymes beyond normal values are sure indicators of liver toxicity (Abdel *et al.*, 2014). Thus, this study aims at determining the hepatotoxicity effects of methanolic-extracts of *Gmelina arborea* leaves on liver marker enzymes in wistar rats

2. Methodology

2.1. Plant Material Sampling

The plant *Gmelina arborea* used for this study was obtained from a farmland in Alike autonomous community in Obowo local government in Imo State, Nigeria. The plant was identified by a taxonomist Prof. G. C. Osuagwu of the Department of Plant Science and Biotechnology, Michael Okpara university of Agriculture Umudike. The leaf of *Gmelina arborea* was separated from its stalk. The leaf was chopped and then air dried under shade for seven days. It was further pulverized into fine powder using a milling machine.

2.2. Plant Material Extraction

Grinded extract was soaked in 98% Methanol for 72 hours with continuous stirring to ensure proper mixing. The liquid part was then squeezed (using muslin cloth) out and filtered using a filter paper, the filtrate was stored in the oven of about 38°C

2.3. Experimental Animal

Healthy male wistar rats of about 120-150g in weight were used for this study. They were obtained from department of Veterinary Medicine, Michael Okpara University of Agriculture Umudike in Abia State, Nigeria. They were left for one (1) week acclimatization in a clean aluminum cage and a well-ventilated animal house of the Department of Biochemistry, Michael Okpara University of Agriculture Umudike, with water and poultry feed pellets.

2.4. Experimental Design/protocol

25 male wistar rats weighing between 120-150g wistar rats were randomly divided into six (5) groups.

Animal Groups	Number	Treatment	Duration
Control	5	Water and feed	21 days
Group 2	5	1000mg/kg of extract per rat twice daily	21 days
Group 3	5	500mg/kg of extract per rat twice daily	21 days
Group 4	5	250mg/kg of extract per rat twice daily	21 days
Group 5	5	150mg/kg of extract per rat twice daily	21 days

2.5. Animal sacrifice and serum preparation

The animals were sacrificed two from each group that was administered every seven days. The sacrifice was done by ocular puncture and abdominal cut. Serum was separated by centrifuging. Serum transaminase was measured for assaying and making group comparisons of liver function.

3. Liver Function Parameters

3.1. Qualitative Estimation of Serum Alanine Transaminase (ALT) and Aspartate Transaminase (AST)

This method is based on the principle that pyruvate is formed when ALT catalyses the reaction between oxoglutarate and alanine. The keto acid formed reacts with 2, 4 - dinitrophenyl hydrazine (2, 4 DNPH). The absorbance of the resultant colour due to the formation of pyruvate hydrazone measured photo metrically at 540nm. ALT is determined by monitoring the concentration of pyruvate hydrazone formed when reacts with 2, 4-dinitrophenyl hydrazine (2, 4 DNPH). For AST, the method is based on the principle of the reaction between oxoglutarate and aspartate catalyze by

AST, resulting to the formation of pyruvate. AST is determined by monitoring the concentration of oxaloacetate hydrazone formed with 2, 4-dinitrophenyl hydrazine (2, 4 DNPH) (Reitman and Frankel, 1957).

3.2. Reagent Composition

The reagent (RANDOX kit) and chemical used includes; phosphate buffer (0.1M ph 7.4), standard solution (2Mm sodium pyruvate), 2, 4-dinitrophenyl hydrazine (1Mm in 1mM HCl), NaOH (0.4N), AST substrate (0.1M L-Aspartate, 2Mm of 2-Oxoglutarate), ALT substrate (0.1 M L-Alanine, 2Mm 2-Oxopyruvate).

3.3. Procedures of Serum Alanine Transaminase (ALT) and Aspartate Transaminase (AST)

The procedures for the determination of AST and ALT are almost similar. Test tubes were labeled blank, standard, control and the test. A volume of 0.5ml of the substrate 2, 4-DNPH (Dinitrophenyl hydrazine) introduced into all the test tubes mixed with 0.1ml of the serum sample or standard added to the test tubes. The mixture was allowed to incubate for 30 mins for ALT and 60 mins for AST at 30. Briefly, 0.5ml of the color reagent was added, mixed and allowed to stand for 20 mins to the reacting mixture; therefore 0.5ml of NaOH was added after 5 minutes and followed by the measurement of absorbance against blank at 540nm.

3.4. Total Bilirubin Test

The total bilirubin activity was assayed using the method described by Reitman and Frankel, 1957.

Principle: Bilirubin reacts with diazotized sulfanilic acid to produce azobilirubin, which has an absorbance maximally at 560nm in the dimethyl sulfoxide (DMSO) solvent. The intensity of the colour produce is directly proportional to the amount of total bilirubin concentration presents in the sample.

3.5. Conjugated bilirubin test

The conjugated bilirubin activity was assayed using the method of Reitman and Frankel (1957) as outlined in Randoz test kit (USA).

Principle: Bilirubin react with diazotized sulfanilic acid to produce azobilirubin, which has an absorbance maximum at 560nm in the aqueous solution. The intensity of the colour produced is directly proportional to the amount of direct bilirubin concentration present in the sample.

4. Statistical Analysis

The data analysis was done using the baseline characteristics expressed as the mean \pm Standard Deviation (SD), One-way analysis of variance (ANOVA) with Duncan test using SPSS software version 23.0 for multiple comparisons between metabolic groups

5. Results and discussion

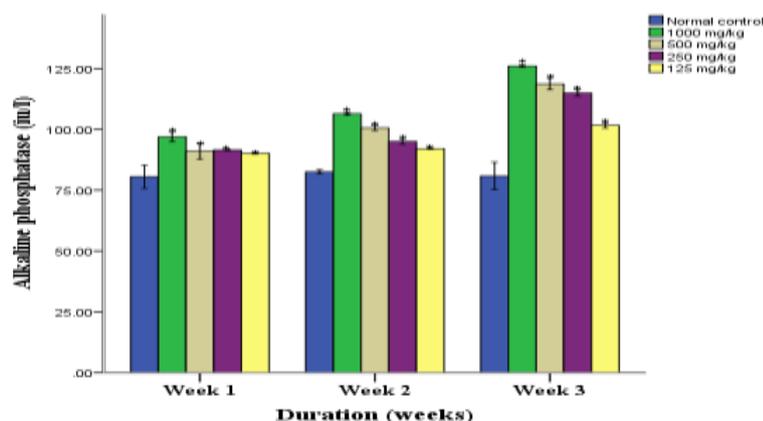


Figure 1 Graphical representation of the effect of Methanol extract of *Gmelina arborea* on alkaline phosphatase concentration

From the above bar chart, there was a significant ($p < 0.05$) increase in all the extract groups when compared to the normal control in week 1, 2, and 3.

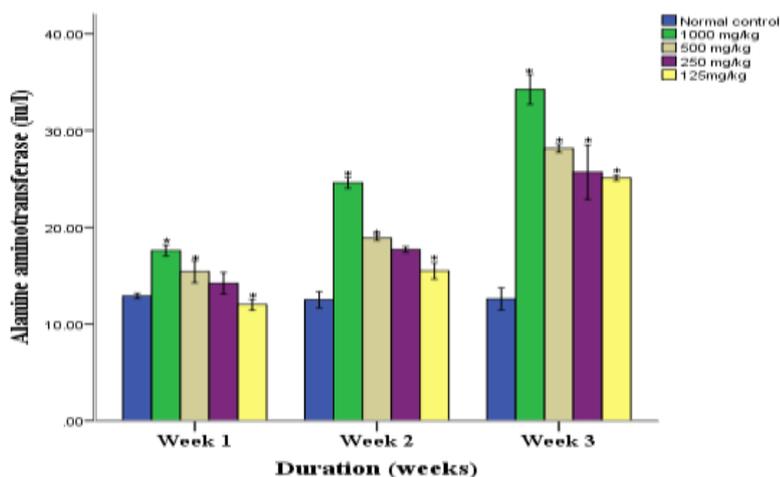


Figure 2 Graphical representation of the effect of Methanol extract of *Gmelina arborea* on Alanine aminotransferase concentration

The above bar chart indicates that there was a significant ($p < 0.05$) increase in 1000 mg/kg, 500 mg/kg and 125 when compared to the normal control in week 1, 2, and 3 but there was no significant ($p < 0.05$) increase in 500 mg/kg in week 1 and 2 when compared to the normal control.

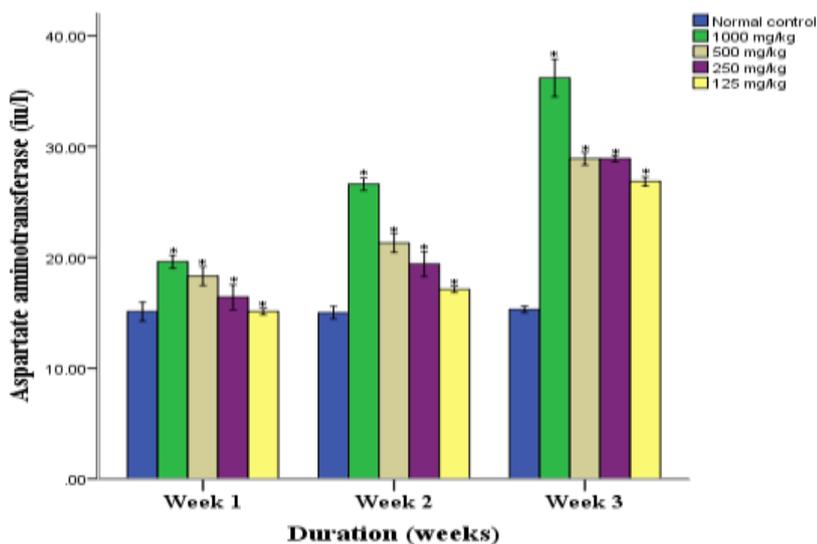


Figure 3 Graphical representation of the effect of Methanol extract of *Gmelina arborea* on Aspartate aminotransferase concentration

The bar chart above result shows that there was a significant ($p < 0.05$) increase in all the extract groups when compared to the normal control in week 1, 2, and 3.

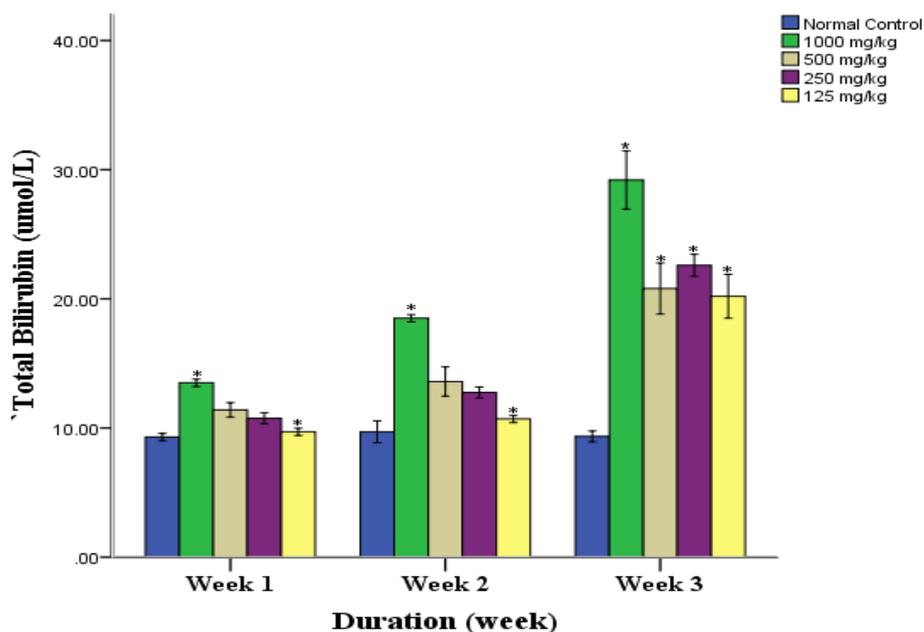


Figure 4 Graphical representation of the effect of Methanol extract of *Gmelina arborea* on Total Bilirubin concentration.

There was a significant ($p < 0.05$) increase in (1000 mg/kg and 125 mg/kg) when compared to the normal control in week 1, 2, and 3 but there was no significant ($p > 0.05$) increase in 500 mg/kg and 250 mg/kg when compared to the normal control in week 1 and 2.

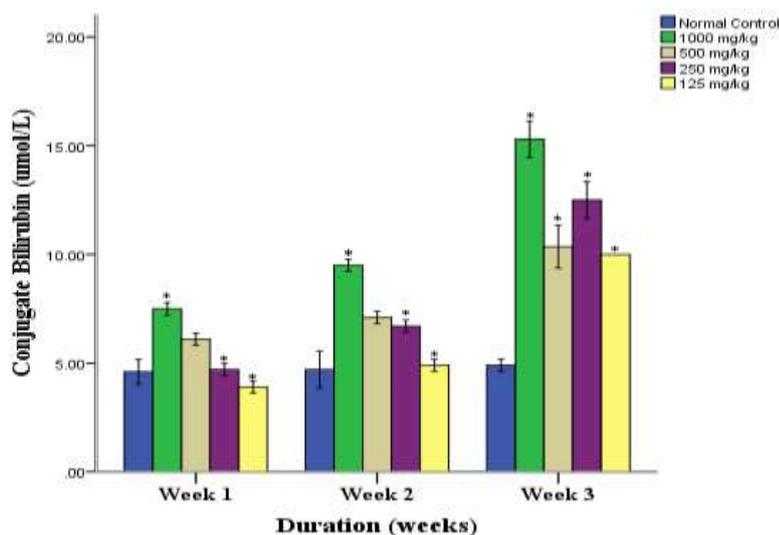


Figure 5 Graphical representation of the effect of Methanol extract of *Gmelina arborea* on Conjugate Bilirubin concentration

The above chart indicate that there was a significant ($p < 0.05$) increase in all the extract groups when compared to the normal control in week 1 and 2.

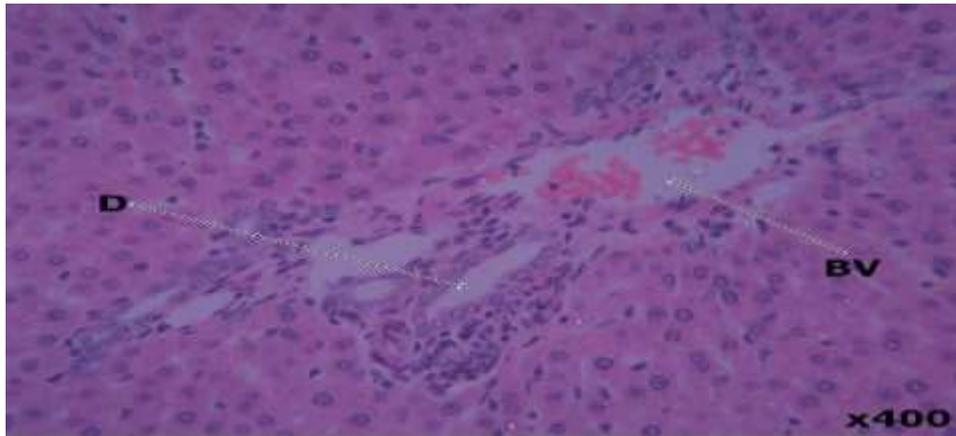


Plate 1 Normal control

Photomicrographs shows well preserved liver architecture triads are evenly spaced around a central vein and there is mild portal inflammation without interface necrosis or fibrosis. There is no steatosis

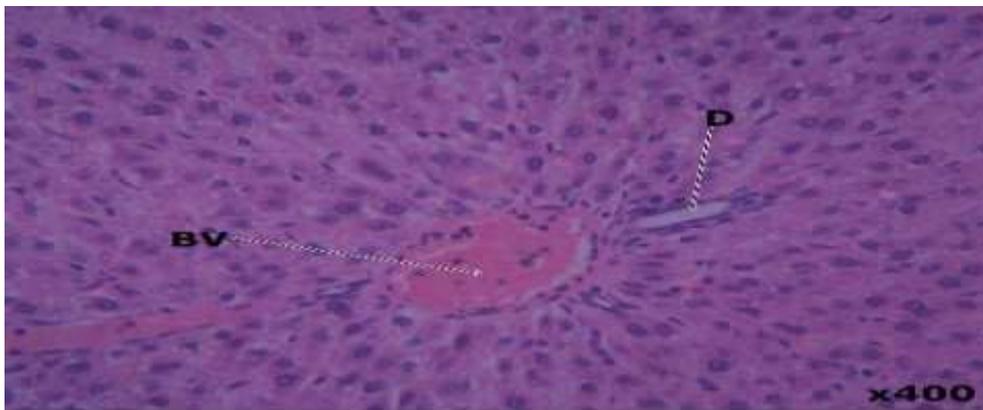


Plate 2 Group 2(1000mg/kg)

Compared to normal control there was no pathology in group 2.

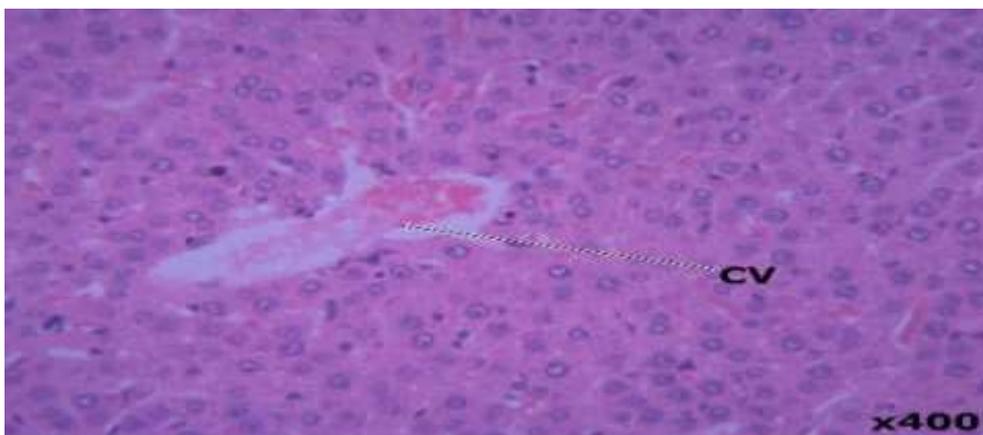


Plate 3 Group (250mg/kg)

Compared to normal control there was no pathology in group 3

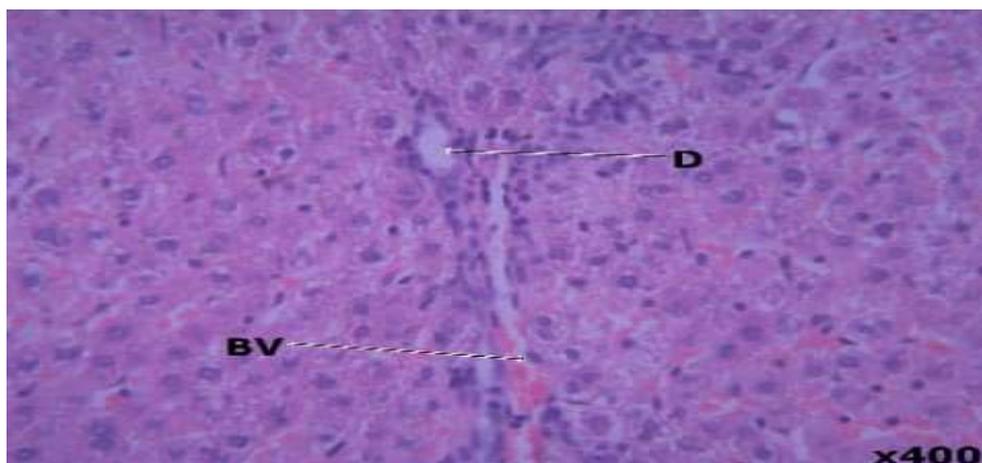


Plate 4 Group 4 (150mg/kg)

Compared to normal control there was no pathology in group 4

6. Discussion

From the obtained results, *G. arborea* did not show any toxic reactions at a dose of 500mg/kg and below. Thus, the no-observed adverse effect level (NOAEL) of ME was 500 mg/kg. In the present study, ME at all selected doses did not show significant changes in body weights as compared to the control group. *G. arborea* did not appear to affect the behaviour of the rats at the administered oral doses of 150, 250, and 500 mg/kg. There were no significant differences in biochemical parameters of the groups treated with ME of *G. arborea* compared to the control. The lack of significant alterations in the levels of ALT, AST, ALP, TB and CB good indicators of liver functions, which suggests that the repeated administration of ME of *Gmelina arborea* do not have toxic effects on liver. Liver function tests give information about the state of the liver, describing its functionality (albumin and lipid profile), cellular integrity (transaminases) and its link with biliary tract (ALP) (Ezejiolor et al., 2013). Thapa and Anuj (2007) had reported that standard range of accepted values for liver function tests, beyond which liver damage may be suspected is ALT (10 – 55 μ /L), AST (10 – 40 μ /L), and ALP (45 – 115 μ /L). Kamal and Hessah (2015) corroborated this when they reported that rise in AST, ALT and ALP values beyond this limits indicate early diagnosis of hepatotoxicity and tissue damage. It has been reported that liver toxicity is associated with increase in various serum liver enzymes resulting from damage to the hepatocytes. The plant has been reported for the presence of phenolics as one of the important phytoconstituents. Plant phenolics are well known for their antioxidant activity. The plant has shown a significant antioxidant activity in *in vitro* models like DPPH, FRAP, and ABTS assay. The phenolics in *G. arborea* may play a protective role against the oxidative damage to the liver cells by scavenging the free radicals.

The histopathological studies of important organs after the administration of ME indicated no alterations in tissue structures. This supports the results from biochemical analysis, and the oral administration of ME at a high dose of 1000 mg/kg/day for 21 days was well tolerated by the treated rats.

7. Conclusion

In conclusion, the methanol extract of *G. arborea* was found to be safe in acute repeated dose toxicities in the rats. There was no mortality observed as a result of administration of this leave extract. Methanol extract of *Gmelina arborea* did not produce significant differences in the body weight, organ weights and feeding habits between control and treated animals. Pathologically, neither gross abnormalities nor histopathological changes were observed. Methanol extract of *Gmelina arborea* was found safe in acute and repeated dose toxicity study when tested in wistar rat.

Compliance with ethical standards

Acknowledgments

This work was carried out in collaboration between the authors: Omodamiro, O.D designed, supervised, performed the analysis and wrote the manuscript of the study. Author Alaabo, P.O assisted in provision of essential materials and collection of samples data. Olukotun. B.G prepared and interpreted the histological slides, while Chikezie, P.C read and approved the final manuscript.

Conflict of interest

Authors declared that no conflict of interest existed in this paper.

Ethical approval and consent to participate

The study was conducted by following the guidelines set by National Institute of Health, USA as approved by the College of Veterinary Medicine, Micheal Okpara University of Agriculture, Umudike. The ethical committee's reference number is: MOUAU/CVM/REC/202015.

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