

AFS2023002/24108

Effect of Aqueous and Methanolic Extract of *Annona muricata* Stem Bark on Liver Enzymes in Normal Sprague Dawley Rats

Olumese, Fidelis E,*¹ Ukuoku, Oghenerukevwe James² and Orhue, Jerry N.²

¹Department of Medical Biochemistry, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City, Edo State, Nigeria.

²Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria.

*Corresponding author Email: fidelis.olumese@uniben.edu Tel: +234 (0) 802 353 8551

(Received March 2, 2023; Accepted in revised form March 5, 2023)

ABSTRACT: The use of plant extracts to treat diseases in herbal medicine has the potential of causing toxic effects, though of natural origin, it may not be safe. This study evaluated the effect of aqueous and methanolic extract of *Annona muricata* stem bark consumption in normal rats. Fresh stem bark of *Annona muricata* (Sour soup) was collected, pulverized, extracted with distilled water or methanol (Aqueous or Methanolic extract). The rats were divided into six (6) groups of five (5) rats per group, for aqueous or methanolic extract. Group 1 (control) rats were administered distilled water, while groups 2, 3, 4, 5 and 6 were administered 200, 500, 1000, 3000 and 5000 mg/kg body weight of aqueous and methanolic extract for 28 days. Liver function indices in the serum and histopathological evaluation of the organ were performed. ALT levels at 200 and 500 mg/kg in aqueous or methanolic extract were significantly ($p < 0.05$) altered. Oral administration of aqueous extract significantly ($p < 0.05$) reduced the concentrations of total bilirubin and increased ($p < 0.05$) total protein in a dose-dependent manner. The study showed that oral administration of aqueous or methanolic extract did not have any deleterious effects on the liver architecture.

Keywords: Plant Bark, Rats, Liver, Methanol, Bilirubin

Introduction

The leaves, root, seeds and stem bark of plants have been used as herbal medicinal remedies to treat diseases by people in various part of the world. Medicinal plants serve as herbal remedies in diverse culture of the world to treat diseases affecting man such as malaria, hyperglycaemia, cardiovascular diseases and cancer (WHO, 2005). Medicinal plants are great economic importance to a nation, because they are source of foreign exchange and means of providing primary health care to people living in rural communities (Adewole and Caxton-Martins, 2006). The use of plant stem bark extracts decoction to treat diseases in rural communities is not entirely safe as herbal remedies (Larbie *et al.*, 2011), which have not been scientifically screened due to lack of adequate information on the preparation and techniques in the application traditionally (Tijani *et al.*, 2013). This herbal remedies may cause toxic effect to vital body organs and enzymes such as the liver and its enzymes; ALT, AST and ALP, because their scientific mechanism of action and dosage has not been proven (Morelli *et al.*, 2000). The use of plant decoction as herbal remedies can be seen with people in rural communities with less economic income in developing countries (Soetan and Aiyelaagbe, 2009).

Annona muricata is a plant that belongs to the *Annonaceae* family which is widely used to treat diseases due to the bioactive compounds present in various part of the plant with little or no consideration to the toxicological

effects of the plant on body organs and enzymes (Ogbonna *et al.*, 2010 and Kim *et al.*, 1998). *Annona muricata* commonly refers to as custard apple tree produces edible fruits called soursop which has a slight acidic taste when ripe. *Annona muricata* is native to the warmest tropical climate areas in South and North America and tropical areas of the world including India, Malaysia and Nigeria (Adewole *et al.*, 2006). *Annona muricata* is an evergreen erect tree reaching 5-8 m in height and features an open, roundish canopy with large, glossy, dark green leaves. The fruit pulp is excellent for making drinks, fruit juice, candy, ice creams shakes and syrup (Wu *et al.*, 1995). The barks, leaves, and roots are used as antispasmodics, hypotensive and sedative (Nwokocha *et al.*, 2012). *Annona muricata* has proven to be effective in the reduction of blood glucose and regeneration of pancreatic islet cells (Suneel, 2015). More recently the use of *Annona muricata* to treat cancer has been effective with the isolation of acetogenins which has a good potential to treat cancer (Chang, 2003). The aim of this study is to ascertain how safe are the extracts on liver cells and enzymes in normal Sprague-dawley rats.

Materials and methods

Plant material and extraction: Fresh stem bark of *Annona muricata* (Sour soup) was obtained from Edo State trade fair garden, Benin City, Edo State, Nigeria. Fresh stem bark was identified and given the voucher number UBH-A356 at the Department of Plant Biology and Biotechnology, University of Benin. The stem bark was washed, cut into small pieces and air-dried and grinded into powder. The grinded stem bark was extracted with; distilled water for aqueous extract and methanolic extract. The aqueous was soaked for 24 hours in distilled water while that of methanol for 72 h. To achieve maximum yield there was intermittent stirring to allow for percolation and maceration. The sample was filtered using two different sizes of filter. The filtrate from methanol was concentrated using a rotary evaporator, after which it was freeze dried using (Coolsafe Superior Touch 95/55-80) freeze dryer, while that of the aqueous extract was concentrated using a freeze dryer. The freeze-dried sample was stored in the freezer at 4 °C ready for use. The weight of aqueous extract after freeze drying was 480 g while that of the methanol was 520 g.

Animals: Sprague-Dawley rats used in this study were procured from the Department of Pharmacology animal house of the University of Benin, Benin City Edo State. They were acclimatized for two weeks at the Animal Unit of the Department of Pharmacology, Faculty of Pharmacy, University of Benin. They were given standard feed pellet diet and water *ad libitum* throughout the duration of the study.

Experimental design: Sixty (60) male Sprague-dawley rats (average body weight 190.49 ± 2.86 g) were divided into two groups of thirty rats (30) for methanol and thirty (30) for aqueous. There was six groups containing five rats each for both aqueous and methanol. Group1 serve as control while groups 2 to 6 served as the test. They were allowed access to standard feed pellet diet and water *ad libitum* on a 12 hour light/12 hour dark cycle. The animals were acclimatized for two weeks before the commencement of the administration of aqueous and methanolic extract. Group 1 served as the control and had access to distilled water while groups 2 to 6 served as the test groups administered different and regulated doses of the extract according to their body weight using gastric gavage as 200 mg/kg, 500 mg/kg, 1000 mg/kg, 3000 mg/kg and 5000 mg/kg respectively. The weight were determined weekly and the extract concentration (doses) calculated. The rats were monitored for 28 days for any signs of mortality or toxicity. At the end of 28 days the animals were sacrificed under chloroform anaesthesia. Blood was collected via cardiac puncture into appropriate containers for biochemical analysis. The liver was excised and kept in buffered formalin for histological studies.

- Group1 (control): Administered distilled water
- Group 2: Administered 200 mg/kg body weight of aqueous or methanol extract
- Group 3: Administered 500 mg/kg body weight of aqueous or methanol extract
- Group 4: Administered 1000 mg/kg body weight of aqueous or methanol extract
- Group 5: Administered 3000 mg/kg body weight of aqueous or methanol extract
- Group 6: Administered 3000 mg/kg body weight of aqueous or methanol extract

Hepatic indices: Alanine aminotransaminase (ALT) and aspartate aminotransaminase (AST) were carried out using the method described by Reitman and Frankel (1957). Alkaline phosphatase was determined using the method of Tietz (1976). Total protein was determined using the method described by Tietz (1995); albumin was determined using the method described by Doumas *et al.* (1971). Total bilirubin and conjugated bilirubin were determined using the method of Jendrassik and Groff (1938).

Histological analysis: The liver was stored in properly labelled plain bottles containing 10% buffered formalin solution as fixative. Following fixation, the tissue was dissected to select appropriate areas for examination. The tissue was then placed in suitably labelled cassettes (small perforated baskets) to segregate them from other specimens. The tissue was dehydrated by immersing it in a series of ethanol (alcohol) solutions of increasing concentration until pure, water-free alcohol is reached, after which the tissue is cleared using xylene as the

clearing agent to remove the alcohol. The tissue was then fixed in paraffin wax and embedded into a block. Thereafter, a mould was filled with molten wax and the tissue placed in it. A cassette was placed on top of the mould, topped up with more wax, and placed on a cold plate to solidify. When this was completed, the block with its attached cassette was removed from the mold and ready for microtomy. Sections of 5 μm thickness were sliced, stained with hematoxylin and eosin and examined using light microscope (Carson, 1997).

Statistical analysis: Statistical analysis of data was carried out using one way analysis of variance (ANOVA) Statistical package for social sciences (SPSS) version 20.0. The analysis data was reported as mean \pm standard error of mean (SEM). Significant difference using the least significance difference (LSD) analysis, to evaluate the differences among groups and for multiple comparisons among different groups was accepted at 95% confidence level of probability i.e. $p < 0.05$.

Results

Result of aqueous extract of Annona muricata stem bark on the liver of normal sprague dawley rats: Figure 1 shows the effects of aqueous extract of stem bark of *A. muricata* on ALT, AST and ALP activities in normal liver of rats. ALT activity of rats administered 200 mg/kg body weight of the extract showed a significant increase ($p < 0.05$) compared with the control. The AST and ALP activity did not show any significant differences when compared with the control ($p > 0.05$).

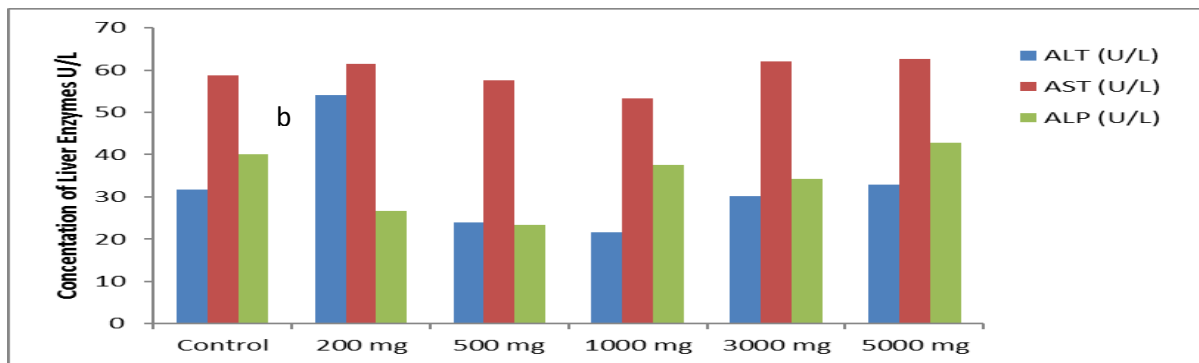


Figure 1: Effect of aqueous extract of *Annona muricata* on ALT, AST, ALP liver enzymes for 28 days. Values are expressed as Mean \pm S.E.M. Values with different alphabets are significantly different from each other at 95% confidence level ($p < 0.05$). $n=5$.

The effect of aqueous extract of stem bark of *A. muricata* on total and conjugated bilirubin is presented in Fig. 2. The total bilirubin activity of the rats administered various doses of the extract demonstrated a significant decrease ($p < 0.05$) when compared to the control while the conjugated bilirubin activity showed no significant difference ($p > 0.05$) when compared to the control.

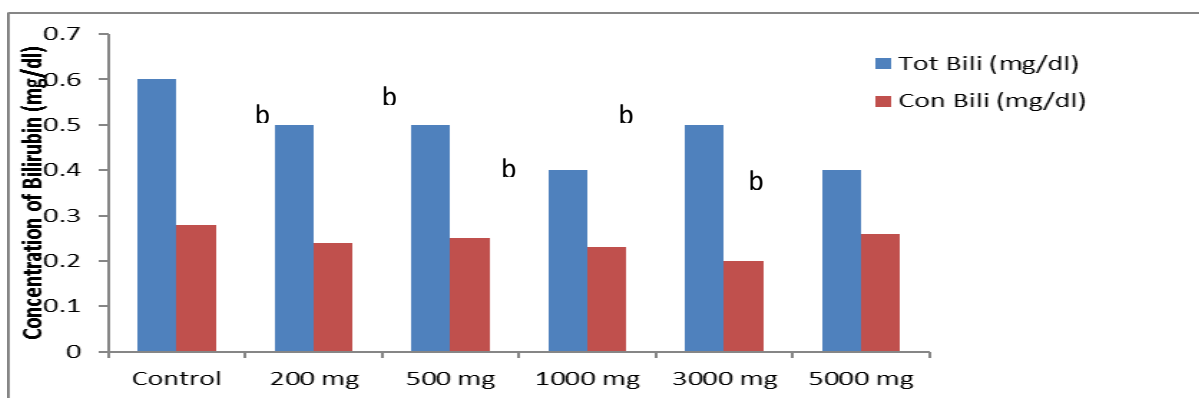


Figure 2: Effect of aqueous extract of *Annona muricata* on total and conjugated bilirubin for 28 days. Tot Bili= Total Bilirubin; Con. Bili= Conjugated Bilirubin Values are expressed as Mean \pm S.E.M. Values with different alphabets are significantly different from each other at 95% confidence level ($p < 0.05$). $n=5$.

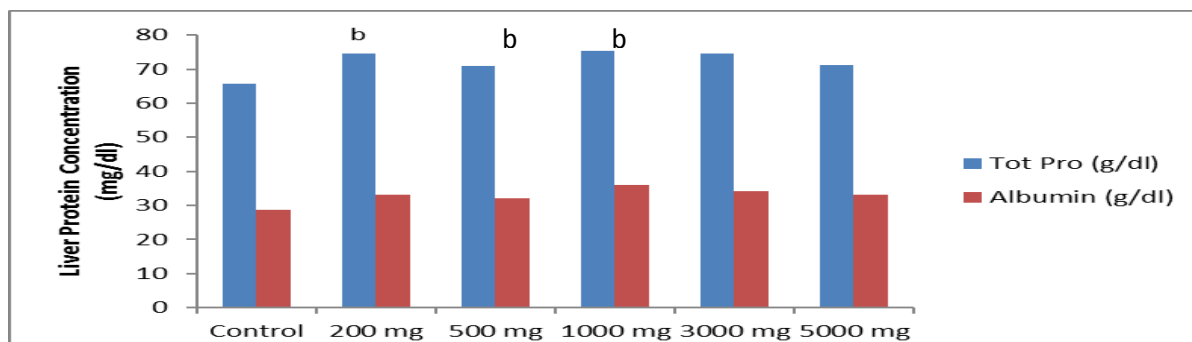


Figure 3: Effect of aqueous extract of *Annona muricata* on total protein and albumin on normal rats for 28 days. Tot. Pro = Total protein. Values are expressed as Mean \pm S.E.M. Values with different alphabets are significantly different from each other at 95% confidence level ($p < 0.05$). $n=5$.

The serum protein activity of rats administered 200mg, 1000mg and 3000mg of aqueous extract showed a significant increase ($p < 0.05$) when compared to normal control (Fig.3). There was no significant changes in the concentration of albumin ($p < 0.05$) when compared to control.

Result of methanolic extract of Annona muricata stem bark on the liver of normal Sprague dawley rats: Fig. 4 shows the effect of methanolic extract of stem bark of *A. muricata* on ALT, AST and ALP activities in rat liver for 28 days. ALT activity of rats administered 200 mg and 500 mg of extract showed a significant increase ($p < 0.05$) compared to the control. The AST and ALP activities showed no significant difference when compared with control ($p > 0.05$).

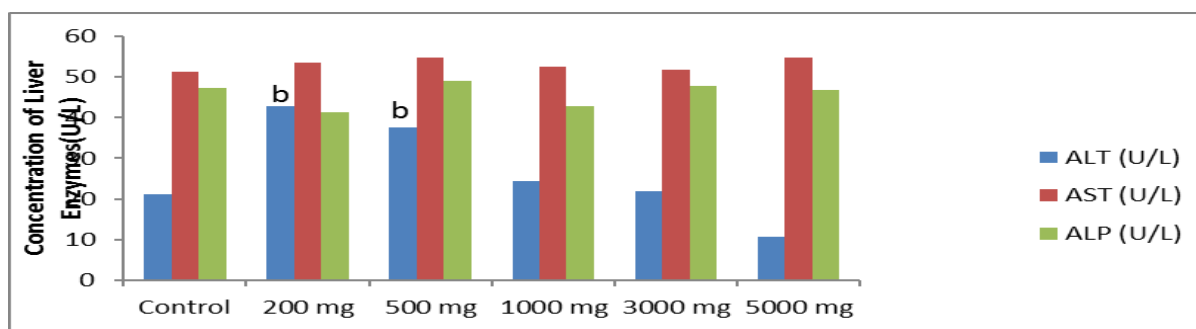


Figure 4: Effect of methanolic extract of stem bark of *A. muricata* on ALT, AST and ALP activities in rat liver for 28 days. Values are expressed as Mean \pm S.E.M. Values with different alphabets are significantly different from each other at 95% confidence level ($p < 0.05$). $n=5$

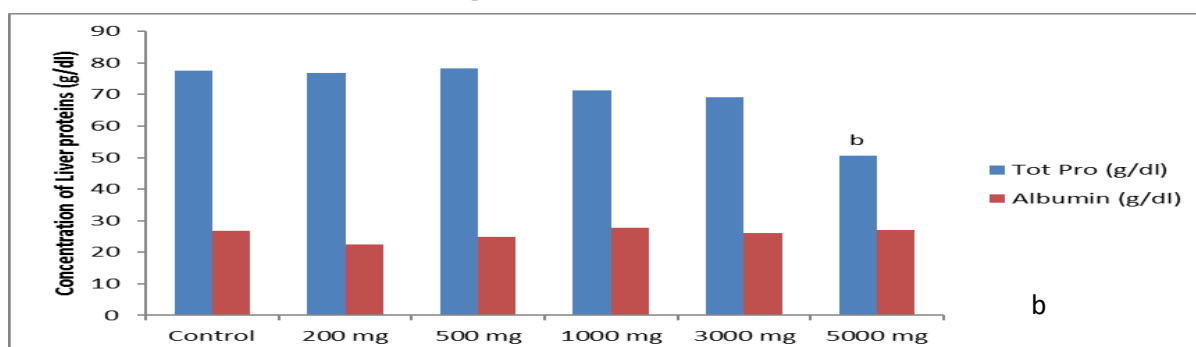


Figure 5: Effect of methanolic extract of stem bark of *A. muricata* on total protein and albumin in rat liver for 28 days. Values are expressed as Mean \pm S.E.M. Values with different alphabets are significantly different from each other at 95% confidence level ($p < 0.05$). $n=5$. Tot Pro=Total protein

The effect of methanolic extract of stem bark of *A. muricata* on total protein and albumin in rat liver for 28 days is shown in Fig. 5. There was a significant decrease ($p < 0.05$) in the concentration of total protein at 5000 mg of extract when compared to control.

The effect of methanolic extract of stem bark of *A. muricata* on total and conjugated bilirubin in rat liver for 28 days is shown in Fig. 6. There was no significant changes ($p > 0.05$) in bilirubin concentrations in the administered doses when compared to control.

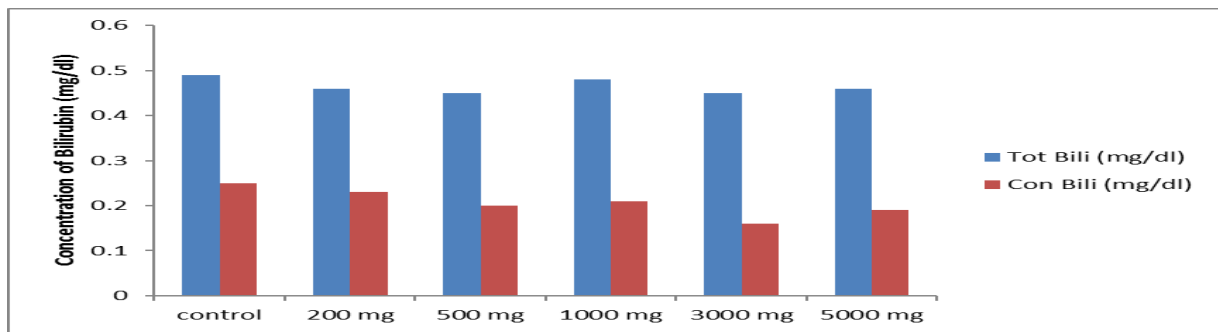
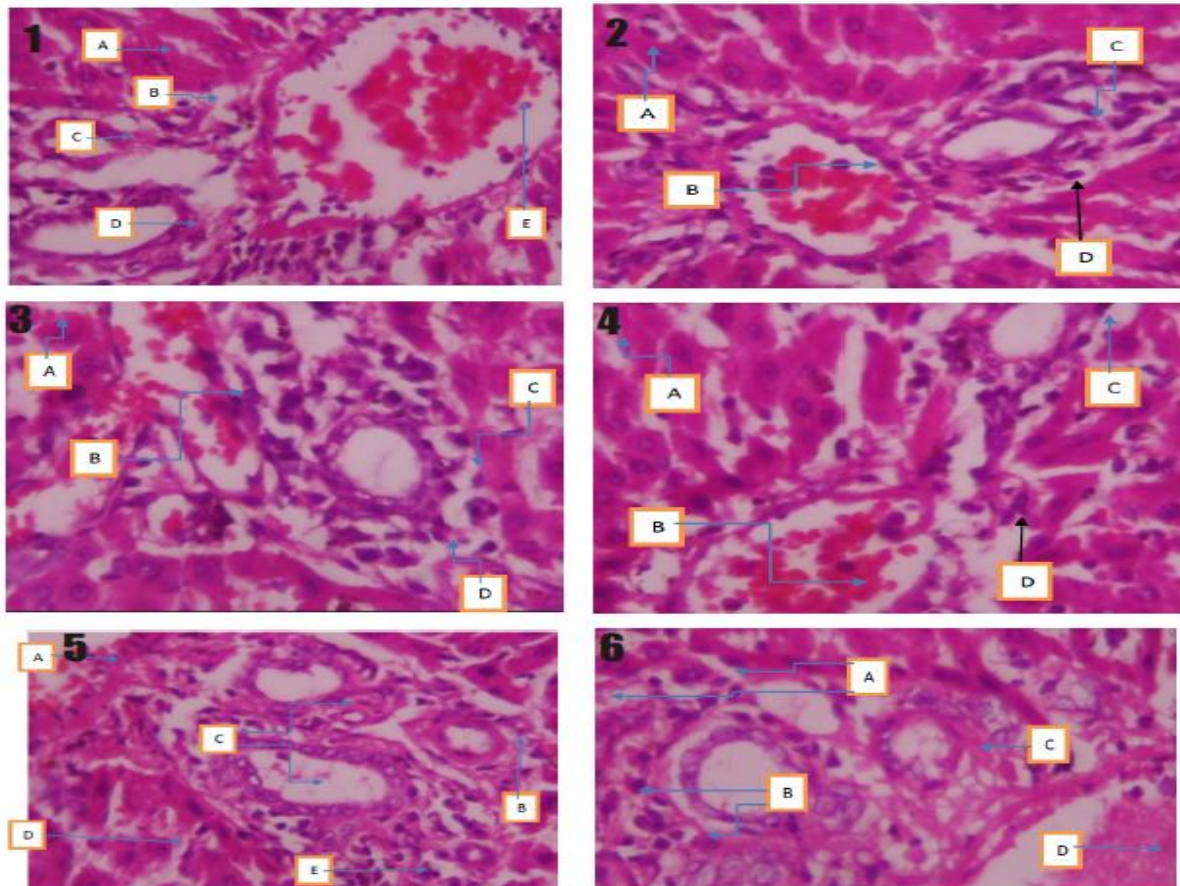


Figure 6: Effect of methanolic extract of *Annona muricata* on total and conjugated bilirubin. Values are expressed as Mean \pm S.E.M. Values with different alphabets are significantly different from each other at 95% confidence level ($p < 0.05$). $n=5$

Photomicrographs of rat liver section stained with H&E after 28 days treatment with aqueous extract of *A. muricata* are shown in Plates 1-6 while Plates 8-12 are photomicrographs of rat liver section stained with H&E after 28 days treatment with methanolic extract of *A. muricata*.



Plates 1-6: Photomicrographs of rat liver section stained with H&E after 28 days treatment with aqueous extract of *A. muricata*. **Plate 1 (Normal control)** Composed of A: normal hepatocytes with inconspicuous nucleoli, B sinusoids, C hepatic artery, D bile duct and E, portal vein. **Plate 2** liver of rat administered 200 mg aqueous extract showed A: normal hepatocytes with inconspicuous nucleoli, B active vascular congestion, C normal bile ducts and D, mild periportal mobilization of lymphocytes. **Plate 3.** Liver of rat administered 500 mg aqueous extract showed A: normal hepatocytes with inconspicuous nucleoli, B active vascular congestion, C normal bile ducts and D, mild periportal mobilization of lymphocytes. **Plate 4.** Liver of rat administered 1000 mg aqueous extract showed A: normal hepatocytes with conspicuous nucleoli, B active vascular congestion, C normal bile ducts and D, mild periportal mobilization of plasma cells. **Plate 5.** Liver of rat administered 3000 mg aqueous extract showed A: mild active portal congestion, B normal hepatic artery, C bile ducts, D normal hepatocytes with inconspicuous nucleoli and E, moderate periportal mobilization of lymphocytes. **Plate 6.** Liver of rat administered 5000 mg aqueous extract showed A: normal hepatocytes with conspicuous nucleoli, B mild periportal mobilization of lymphocytes, C normal bile duct, D portal vascular dilatation and active congestion (Magnification x 400)

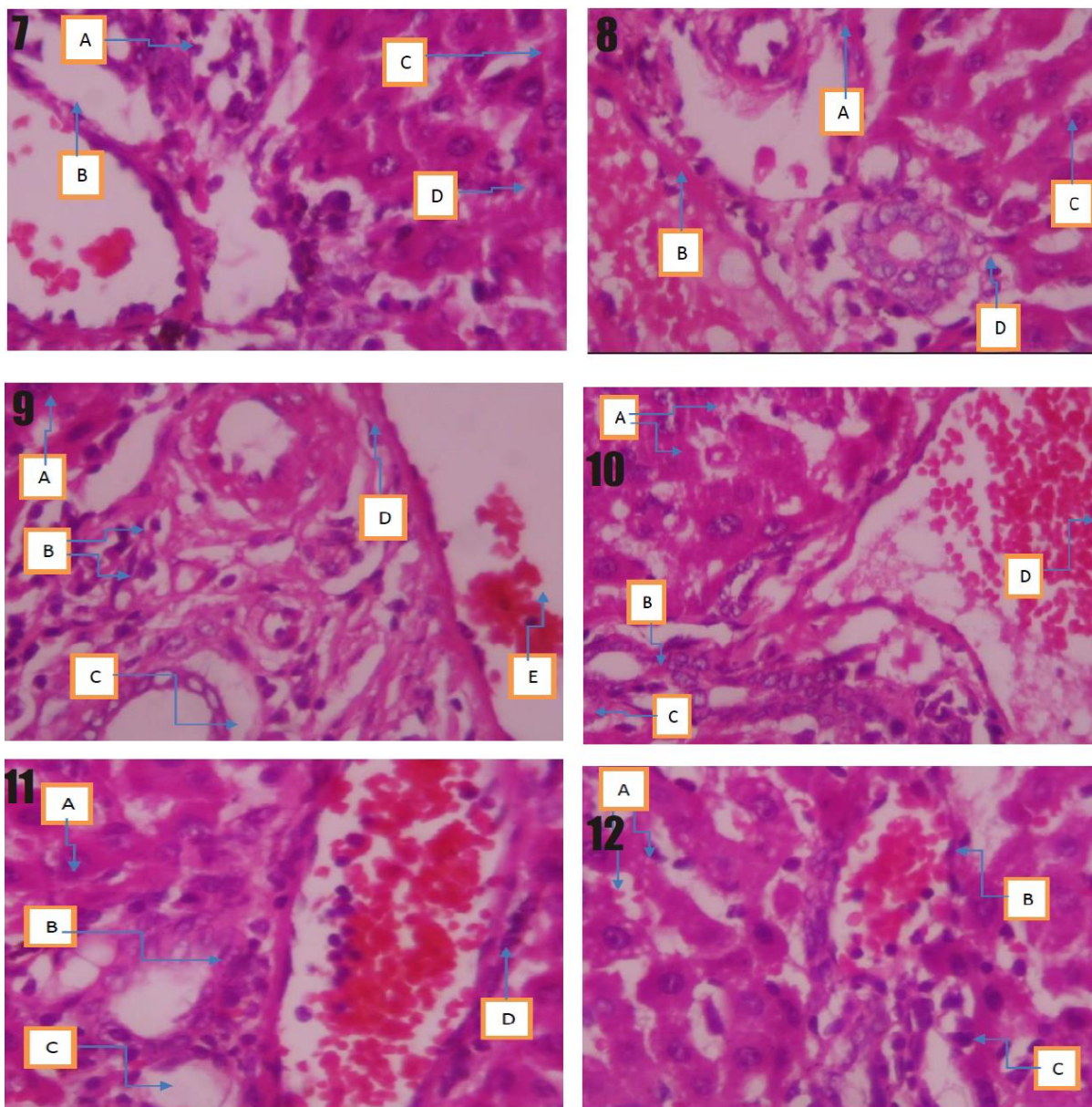


Plate 8-12: Photomicrographs of rat liver section stained with H&E after 28 days treatment with methanolic extract of *A. muricata*. **Plate 7 (Normal control).** Rat liver composed of: A, normal bile duct, B portal vein, C hepatocytes with nuclei containing inconspicuous nucleoli and D, sinusoids. **Plate 8.** Liver of rat administered 200 mg methanolic extract showing: A, normal hepatic artery, B portal vein with active congestion, C, hepatocytes with inconspicuous nucleoli and D, bile duct. **Plate 9.** Liver of rat administered 500 mg methanolic extract showing: A, normal hepatocytes with conspicuous nucleoli, B mild periportal mobilization of lymphocytes, C normal bile duct, D hepatic artery and E, portal vasodilatation. **Plate 10.** Liver of rat administered 1000 mg methanolic extract showing: A, normal hepatocytes with conspicuous nucleoli, B normal bile ducts, C mild periportal mobilization of lymphocytes and D, portal vasodilatation and active congestion. **Plate 11.** Liver of rat administered 3000 mg methanolic extract showed: A, normal hepatocytes with inconspicuous nucleoli, B normal bile ducts, C mild periportal mobilization of lymphocytes and D, portal vasodilatation and active congestion. **Plate 12.** Liver of rat administered 5000 mg methanolic extract showing: A, normal hepatocytes with conspicuous nucleoli, B active portal vascular congestion and C, mild periportal mobilization of lymphocytes (Magnification x 400).

Discussion

In this study we found that *Annona muricata* stem bark plant is safe for consumption as there was no toxic effects and mortality in the rats. The administered aqueous or methanolic extract was well handled by the liver in this study as there were no untoward metabolic changes.

Plants are used as herbal remedies for the health benefits derived from them due to their therapeutic effect (Bailey and Day, 1989). Medicinal plants are used as herbal remedies for various diseases and this is known to be popular in diverse culture of the world and most especially in Africa traditional medicines.

The use of medicinal plants as herbal treatment for various diseases affecting man in various culture and developing countries is of great concern as this could cause toxic harm to vital body organs and enzymes because their effective dosage and mode of action has not been ascertain (Keen *et al.*, 1994). *Annona muricata* stem bark is a plant used widely for treatment of various diseases and herbal decoction is used to treat several diseases (Kedari and Khan2014). This study showed that the aqueous or methanolic extract altered the concentration of ALT at 200 and 500 mg/kg body weight. Alanine aminotransferase (ALT) catalyze the transfer of amino groups to form the hepatic metabolite oxaloacetate ALT is found abundantly in the cytosol of the hepatocyte. In the case of hepatocellular injury or death, release of ALT from damaged liver cells increases measured ALT activity in the serum. It is also found in the kidney, and, in much smaller quantities, in heart and skeletal muscle cells (Crook, 2006). It has been reported that elevation of ALT maybe due to acute hepatotoxicity which decreases on prolong administration of the intoxicant on the liver (Obi *et al.*, 2004). In this study the observed elevation may be as a result of initial response to foreign substance by the liver. Nevertheless, on prolong use of the extract for 28 days there was no elevation of ALT even at higher doses. The histopathological evaluation of the liver did not reveal any deleterious effects.

Aspartate aminotransferase (AST) is one of a group of enzymes which catalyzes the interconversion of amino acids and keto acids by the transfer of amino groups. They are widely distributed in body tissues with significant amounts found in the heart and liver, lesser amounts are also found in skeletal muscles, kidneys, pancreas, spleen, lungs, and brain. Injury to these tissues results in the release of the AST enzyme into circulation (Wilkinson, 1976). This study did not show any significant changes in the AST level in aqueous or methanolic administration of the extract as the microarchitecture of the liver did not reveal any untoward effect. Alkaline phosphatase is a hydrolase enzyme which removes phosphate groups from many types of molecules. Alkaline phosphatase is present in all tissues throughout the entire body, but is particularly concentrated in liver, bile duct, kidney, bone and placenta. Abnormal level of ALP is caused by a number of conditions. This study revealed no significant increase in ALP level in aqueous or methanolic extract of *Annona muricata* stem bark.

Bilirubin accumulates from the breakdown of haemoglobin present in red blood cells. During normal function, the liver removes bilirubin from the blood and excretes it through bile as bile pigment. Increase in total bilirubin levels may indicate a compromise in the liver's ability to excrete bilirubin or an obstruction in the bile duct (Veena *et al.*, 2011). The results showed total bilirubin were significantly decreased ($p < 0.05$) at 200, 500, 1000, 3000 and 5000 mg/kg in the aqueous extract administration and that of the methanolic extract showed a non-significant difference ($p > 0.05$) when compared to the control. Albumin and conjugated bilirubin in both the aqueous and methanolic extracts showed a non-significant difference ($p > 0.05$). This is an indication that the liver was not toxicologically affected during the subchronic administration of *Annona muricata* stem bark aqueous or methanolic extract.

Proteins are important dietary macronutrient required for life (Wolfe *et al.*, 2017) which has various metabolic and physiologic functions, including the regulation of appetite, food intake, body weight, and body composition (Greco *et al.*, 2017). There are a variety of proteins from a wide spectrum of food sources such as meat, milk, egg, soy, and other plants. Animal foods have higher protein to energy ratios and better digestibility of protein and amino acids than plant (Wu *et al.*, 2014). It has been reported that any change in the concentration of serum protein indicates a change in the normal liver functions (Ahmed *et al.*, 1992). In this study the concentration of total protein at 200, 1000 and 3000 mg/kg body weight in the group of rats administered aqueous extract of *A. muricata* were significantly increased ($p < 0.05$) when compared to control, which implied that the synthetic machinery of liver in the rats were not affected. Lymphocytes represent an important component of innate immunity, which is the initial rapid response to potentially dangerous stimuli (Barrington *et al.*, 2005). Histological evaluation of the liver in this study revealed increased activities in the blood vessels of the liver. Histopathological evaluation further revealed that there was mobilization of lymphocytes that is associated with important components of innate immunity. Localization of these components in the liver suggests a central role it plays in systemic as well as regional defence.

Conclusion

This study revealed *Annona muricata* stem bark has innate immunity ability and mobilization potential of lymphocytes for defence against dangerous stimuli as observed in this animal model.

Acknowledgement

We appreciate Mr Philip Akugbe Obarisiagbon, Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin for his technical support

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. All authors read and approved the manuscript for publication.

References

- Abdullah FL, Chua IS, Rahmat Z: Comparison of protein extraction methods for the leaves of *ficus deltoidea*. J Fundam. Appl Sci, 9 (2): 908 – 924. 2017.
- Ahmed M, Saeed MA, Alam H, Ashgar Z: Biological studies of indigenous medicinal plants II: Effects of *Aplotaxis lappa Dcne*. on various parameters of liver metabolism in rabbits. Med J Islamic World Acad Sci, 5(1): 61-66. 1992.
- Adewole SO, Caxton-Martins, EA: Morphological changes and hypoglycemic effects of *A. muricata* Linn. (Annonaceae) leaf aqueous extract on pancreatic B-cells of Streptozotocin-treated diabetic rats. Afri J Biomed Res, 9:173-180. 2006.
- Arthur FKN, Woode E, Terlabi EO, Wu FE, Gu ZM, Zeng L, Zhao GX, Zhang Y, McLaughlin JL: Two new cytotoxic monotetrahydrofuran Annonaceous acetogenins, anomuricins A and B, from the leaves of *Annona muricata*. J Nat Prod, 58: 830-836.
- Agu KC, Okolie NP, Eze I, Anionye JC, Falodun A: Phytochemical analysis, toxicity and hemomodulatory properties of *Annona muricata*. Egypt J Haematol, 42: 36-44. 2017
- Bailey CJ, Day C: Traditional plant medicines as treatment for diabetes. Diabetes Care, 12: 553-564. 1989.
- Berrington JE, Barge D, Fenton AC, Cant AJ, Spickett GP: Lymphocyte subsets in term and significantly preterm UK infants in the first year of life analyzed by single platform flow cytometry. Clin Exp Immunol, 140: 289-292. 2005.
- Carson FL: Histotechnology: A Self-Instructional Text. 2nd Edition, American Society for Clinical Pathology Press, Chicago, p.160. 1997.
- Chang FR, Liaw CC, Lin CY, Chou CJ, Chiu HF, Wu YC: New adjacent Bis-tetrahydrofuran annonaceous acetogenins from *Annona muricata*. Planta Med, 69(3):241-246. 2003.
- Chang FR, Wu YC: Novel cytotoxic annonaceous acetogenins from *Annona muricata*. J Nat Prod, 64(7):925-931. 2001.
- Crook MA: Clinical Chemistry and Metabolic Medicine. 7th edn. Hooker Arnold, London, p. 426. 2006
- Doumas BT, Watson WA, Biggs HG: Albumin standards and the measurement of serum albumin with bromocresol green. Clin Chem Acta, 31(1):87-96. 1971.
- Fahad A: Extraction of protein from common plant leaves for use as human food. Am J Applied Sci, 7 (3): 331- 342. 2010.
- Green RM, Flamm S: AGA technical review on the evaluation of liver chemistry tests. Gastroenterology, 123 (4):1367-1384. 2002.
- Greco E, Winquist A, Jacob LT, Collins S, Lebovic Z, Zerbe-Kessinger T, Jahan MA: The role of source of protein in regulation of food intake, satiety, body weight and body composition. J Nutr Health Food Eng, 6 (6): 186-193. 2017.
- Hauwa BS, Gabi B, Sadiya MA: Acute and subchronic toxicity profile of *Annona Muricata* on wister albino rats. Bajopas, 10 (2): 57-63. 2017.
- Jendrassik L, Grof P: Simplified photometric methods for the determination of bilirubin. Biochem J, 297: 81-89. 1938.
- Kedari TS, Khan AA: Guyabano (*Annona Muricata*): A review of its traditional uses phytochemistry and pharmacology. Am J Res Com, 2(10): 247-268. 2014.
- Keen RW, Deacon AC, Delves HT, Moreton JA, Frost PG: Indian herbal remedies for diabetes as a cause of lead poisoning. Postgrad Med J, 70 (820): 113-114. 1994.
- Kim GS, Zeng L, Alali F, Rogers LL, Wu FE, McLaughlin JL, Sastrodihardjo S: Two new mono-tetrahydrofuran ring acetogenins, anomuricine and muricapentocin, from the leaves of *Annona muricata*. J Nat Prod, 61 (4): 432–436. 1998.
- Kumar S, Pandey AK: Chemistry and biological activities of flavonoids: an overview. Sci World J, 162750. 2013.
- Larbie C, Arthur FKN, Terlabi EO, Woode E: Evaluation of acute and subchronic toxicity of *Annona muricata* (Linn.) aqueous extract in animals. Eur J Exp Biol, 1(4), 115–124. 2011.

- Morelli V, Zoorob RJ. Alternative therapies: Part I. Depression, diabetes, obesity. *Am Fam Physician*. Sep 62(5):1051-60. 2000. PMID: 10997530.
- Nwokocha CR, Owu DU, Gordon A, Thaxter K, McCalla G, Ozolua RI, Young L: Possible mechanisms of action of the hypotensive effect of *Annona muricata* (soursop) in normotensive sprague-dawley rats. *Pharm Biol*, 50(11): 1436–1441. 2012.
- Obi E, Orisakwe OE, Asomugha LA, Udemezue OO, Orish VN: The hepatotoxic effect of halofantrine in guinea pigs. *Indian J Pharmacol*, 36(5):303-305. (2004).
- Reitman S, Frankel S: Colorimetric method for the determination of serum glutamic oxaloacetylase aminotransferase. *Am J Clin Pathol*, 28: 56-63. 1957.
- Soetan KO, Aiyelaagbe OO: The need for bioactivity- safety evaluation and conservation of medicinal plants – A review. *J Med Plant Res*, 3(5): 324–328. 2009.
- Suneel KA, Venkatarathanamma V, Naga SV, Seetha RK: Antipyretic activity of *Annona* plants leaves on brewer's yeast induced febrile rats. *Asian J Pharm Clin Res*, 8 (3): 210–212. 2015.
- Tietz NW: *Fundamentals of clinical chemistry* (2nd ed.) W.B Saunders Co, Philadelphia. pp 874-897. 1976.
- Tijani MA, Abdurrahman FI, Abba YS, Idris M, Baburo BS, Mala GA, Dungus MHM, Aji BM, Abubakar KI: Evaluation of Proximate and Phytochemical Composition of Leaves of *Annona senegalensis Pers* . *J Pharm Sci Innov*, 2(1): 7-9. 2013.
- Usunobun U, Okolie NP: *Annona muricata* prevent hepatic fibrosis by enhancing lysosomal membrane stability and suppressing extracellular matrix protein accumulation. *Int J Med*, 4 (1): 10-13. 2016.
- Usunobun U, Okolie NP: Effect of *Annona muricata* pretreatment on liver synthetic ability, kidney function and haematological parameters in dimethylnitrosamine administered rats. *Int J Med*, 4 (1): 1-5. 2016.
- Usunobun U, Okolie NP, Anyanwu OG, Adegbeji AJ, Egharevba ME: Phytochemical screening and proximate composition of *Annona muricata* leaves. *Eur J Bot Plant Sci Phytol*, 2 (1): 18-28. 2015.
- Veena S, Ritu BV, Shatruhan S: Preliminary evaluation of the hepatic protection by pharmacological aqueous extract of *Asparagus racemosus* in lead loaded swiss albino mice. *Int J Pharm Pharm Sci*, 4(1): 55-62. 2011.
- Wilkinson JH: *The Principles and Practice of Diagnostic Enzymology*. Pp.Xvi +592, illustrated. Edward Arnold. London. 1976.
- World Health Organization (WHO). Preventing chronic disease, a vital investment: WHO global report. World Health Organization. Available at: <https://apps.who.int/iris/handle/10665/43314>. 2005. Accessed 3rd February, 2023.
- Wolfe RR, Baum JI, Starck C, Moughan PJ: Factors contributing to the selection of dietary protein food sources. *Clin Nutr*, 37 (1): 130–138. 2017.
- Wu FE, Gu ZM, Zeng L, Zhao GX, Zhang Y, McLaughlin JL, Sastrodihardjo S: Two new cytotoxic monotetrahydrofuran Annonaceous acetogenins, annomuricins A and B, from the leaves of *Annona muricata*. *J Nat Prod*, 58: 830-836. 1995.