Biochemical and Toxicological Evaluation of Stem Bark Extract of *Annona muricata* in Male Wistar Rat

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Abstract

This study was designed to evaluate the biochemical and toxicological properties of stem bark extract of Annona muricata in twelve (12) male Wistar rats, weighing 190-220g. The rats were grouped into three (3), A-C. Group A served as normal control while groups B and C were given different graded doses of methanol extract of Annona muricata (MEAM) stem bark orally (1000mg/kg and 2000mg/kg respectively) at once daily for 21 days. 5mls of blood were collected aseptically for the determination of alanine transaminase (ALT), aspartate transaminase (AST), total white blood cell counts (TWBCC), packed cell volume (PCV), hemoglobin (Hb) concentration, and creatinine levels.

There was no statistically significant difference ($P<0.05$) in ALT, TWBCC, PCV, Hb, and creatinine in groups B and C when compared to control. However, there was a significant increase ($P<0.05$) in the level of AST in group C when compared to the control. In conclusion, the stem bark extract of Annona muricata has no adverse effect. Hence, it is safe when administered in a graded dose.

Keywords: *Annona muricata; Alanine transaminase; Aspartate transaminase; Haemoglobin, creatinine*

Introduction

Medicinal plants are globally regarded as the basis for health preservation and care. Diseases such as cancer, diabetes, and cardiovascular problems have reached epidemic proportions and are viewed as severe health problems; consequently, the therapies for these diseases are of clinical importance (WHO, 2005). *Annona muricata* L. is a plant of the Annonaceae family that has been actively explored in the last decades due to its therapeutic potential. The therapeutic applications of the Annonaceae family were described a long time ago (Billo´n, 1869), and since then, this plant has drawn attention due to its bioactivity. Fruit juice and infusions of leaves or branches have been used to treat fever. *Muricata* is known as soursop (English), Graviola (Portuguese), and Guana´bana (Latin American Spanish). The fruit is not only enjoyed as food but the juice is utilized as a galactagogue to cure diarrhea, and heart and liver problems (Badrie and Schauss, 2009; Hajdu and Hohmann, 2012). The therapeutic uses of A. muricata leave included treatments for hypertension (Badrie and Schauss, 2009; Ezuruike and Prieto, 2014), diabetes (Ezuruike and Prieto, 2014), and cancer (Monigatti *et al.*, 2013). Some patients employed decoctions or capsules of A. *muricata* for cancer and pharmacological therapy (Tisott *et al.*, 2013).

Two hundred and twelve active ingredients have been reported to be discovered in A. muricata (Ana *et al.*, 2018). The major chemicals are acetogenins followed by alkaloids, phenols, and other substances. Leaves and seeds are the principal plant organs researched, perhaps because they are the most traditionally used (Yahaya *et al.*, 2017). Thirty-seven phenolic compounds are present in A. muricata (Ana *et al.*, 2018). The main phenolic chemicals discovered in A. muricata leaves include quercetin (Nawwar *et al.*, 2012) and gallic acid (Correa-Gordillo *et al.*, 2012). The presence of flavonoids and lipophilic antioxidant chemicals such as tocopherols and tocotrienols has been observed to be present in the pulp (Correa-Gordillo *et al.*, 2012). In different investigations, when organic or aqueous extracts have been used, the quantity of extractable total phenols is greatly variable. This is crucial to emphasize the most common therapeutic usage is aqueous infusion because the majority of phenols are soluble in water. Phenolic compounds are considered the principal phytochemicals responsible for the antioxidant action (George *et al.*, 2014). These phytochemicals have shown pharmacological activities such as antimicrobial, antioxidant, insecticide, larvicidal, selective cytotoxicity to tumoral cells, anxiolytic, anti-stress, anti-ulceric, wound healing, anti-jaundice, hepatoprotective, hypoglycemic, immunomodulatory, and antimalarial among others (Yahaya *et al.*, 2017). Many novel phytochemicals are also yet to be found in A. *muricata*. Mechanisms of action of the plant extracts and phytochemicals against toxicity have been proposed and they include disruption of mitochondrial membrane to stop cells in G0/G1 phase, the induction of apoptosis, the blockage of numerous signaling pathways that govern metabolism, induction of metastasis, and necrosis of cancer cells. The mechanism of action of antioxidant activity is through hydrogen donation (Yahaya *et al.*, 2017).

Many herbal therapies have identifiable medicinal effects but may also have harmful side effects. Although medicinal plants are typically believed to be harmless, they are not fully free of side effects or toxicity (Boukandou *et al.*, 2015). The toxicity of medicinal plants varies with the chemical composition of the plant in question. Despite the importance of medicinal plants, some may be a hazard to the health of the users due to potential negative effects or side effects that may be associated with overdose or toxic principles. This may lead to toxicity and mortality in patients (Schultz *et al.*, 2020), so this work is aimed to assess the biochemical and toxicological features of stem bark methanolic extract of Annona muricata in Wistar rats.

Materials and Methods

Animals

This study was carried out on healthy and young adult male Wistar rats weighing 190-220g. The rats were obtained from the Veterinary Research Institute, Vom, Jos, Nigeria. The animals were kept under standard housing conditions of light, temperature, and humidity. They were given free access to food (standard pellets) and drinking water ad libitum.
The National Research Council's Guide for the Care and Use of Laboratory Animals (Research, 1996) was adhered to.

Plant Collection, Authentication, and Preparation

The fresh stem bark of *Annona muricata* was collected from Awgu town in Enugu State, Eastern Nigeria. It was identified and authenticated in the Department of Plant Biology, University of Nigeria, Nsukka, Nigeria. It was air-dried at room temperature. It was milled into fine powder. The powder (1.5kg) was macerated in 5 liters of methanol and extracted at room temperature for 48 hours. The extract was filtered through a Whatman No 1 filter paper and evaporated to dryness in a rotary filtrate evaporator. The crude plant extract (20g) was weighed and dissolved in 100mls of distilled water for use in the experiment at a concentration of 200mg/ml.

Experimental Protocol

The rats were divided into three groups of four rats each; group A (normal control), groups B and C were given different doses of MEAM's stem bark orally (1000mg/kg and 2000mg/kg body weights respectively) at once daily for 21 days.
At the end of 21 days, 5mls of blood was collected from the animals by the retro-orbital puncture. 2ml of each blood sample was dispensed into ethylenediaminetetraacetic acid (EDTA) bottles for immediate determination of PCV, Hb, and TWBC. The remaining volume of blood sample was put in a plain test tube, allowed to clot, and centrifuged at 3000rpm for 5 minutes for maximum extraction of clear sera. The serum samples were stored frozen until analyzed for ALT, AST, and creatinine.

Liver Marker Enzymes Determination

Alanine transaminase and aspartate transaminase

Alanine transaminase and aspartate transaminase were analyzed using the endpoint techniques of Reitman and Frankel (1975) provided by Randoxyl laboratory limited, United Kingdom.
Two test tubes labeled test and blank were set up for the test. 0.5mls of the substrate (ALT/AST) was added to the test tubes and warmed at 37^0C for 5 minutes, and 0.1ml of serum was added to the test tube labeled test and incubated at 37^0C for 60 minutes and 30 minutes for ALT substrate respectively. 0.5ml of 2, 4 –DNPH was added to the two test tubes at the end of the incubation period. 0.1ml of serum was added to the blank sample only. The two tubes were then removed from the water bath and incubated at room temperature for 20 minutes. 5mls of NaOH were added to the two samples respectively. The absorbance (A) was read at 540nm and the result was read from a standard curve.

Determination of Creatinine

The modified Jaffe method of Blass *et al*. (1974) was used for this determination. The protocol in the QCA test kit for creatinine was used. Creatinine reacts with picric acid in an alkaline medium to form a red-colored complex measured at 500nm.

1ml of serum was pipette into a test tube for the test, and 0.5ml of the working standard was pipette into another tube; this is the low standard. 1ml of working standard was again pipetted into another tube; this is a high standard.
1ml, 1.5ml, and 2mls of distilled water were added to the test, low standard, high standard, and blank tube respectively. 1ml 5% sodium tungstate was added to each of the 4 tubes respectively. 1ml of 0.6 NH2SO4 each was added to each of the tubes respectively. The solutions were mixed thoroughly and allowed to stand for 10 minutes. They were centrifuged at 3000rpm for 5 minutes. Supernatants were collected and the following additions were made to the corresponding labeled tubes as indicated below:
They were mixed well and allowed to stand for 15 minutes, their extinction was measured in the colorimeter at 500nm against the blank.

Haemoglobin, Packed Cell Volume, and Total White Blood Cells Count Estimation
The Hb content of the blood was determined by using Sahli's hemoglobinometer methods, PCV was estimated using the microhaematocrit method and WBC count was done using a standard procedure (Bain *et al.,* 2006). Blood was diluted to 1: 200 with Hayem's fluid which preserved the corpuscles and then counted with a Neubauer counting chamber under a light microscope.

Statistical Analysis
The results were expressed as mean ± standard error of the mean. Data were analyzed with one-way analysis of variance from statistical package for social sciences (SPSS) version 15 followed by Dunnet *post hoc* comparison. Mean values of test groups were compared with those of the control groups and regarded as significant at $p<0.05$.

Results
From table 1, there was no statistical significant difference ($P<0.05$) in ALT in group B and C when compared to control. However, there was a significant difference ($P<0.05$) in the level of AST in group C when compared to control.
Table 1: Values of ALT and AST of methanolic extract of *Annona muricata* (MEAM) groups and control group.

Groups	ALT (ui/L)	AST (ui/L)
Group A (Normal control)	18.5±3.12[a]	32.00±4.55[a]
(Group B) 1000mg/kg MEAM treated	19.75±2.87[a]	39.00±2.94[a]
(Group C) 2000mg/kg MEAM treated	21.75±4.35[a]	40.00±2.45[b]

Means with the same letter in same row are not significantly different ($p<0.05$). Values are expressed as mean±SEM for 5 rats in each group. ALT, Alanine transaminase; AST, Aspartate transaminase.

In table 2, there was no significant difference ($P<0.05$) in the values of TWBC, PCV, and Hb, in group B and C when compared to control.

Table 2: Values of TWBC, PCV and Hb of methanolic extract of *Annona muricata* (MEAM) groups and control group.

Groups	TWBCC (cu.mm)	PCV (%)	Hb (gm/L)	Creatinine (Umol/L)
Group A (Normal control)	4125.00±1155.289[a]	48.25±2.99[a]	15.90±0.96[a]	95.75±9.39[a]
(Group B) 1000mg/kg MEAM treated	6450.00±3158.59[a]	47.75±3.86[a]	15.75±1.31[a]	112.00±32.49[a]
(Group C) 2000mg/kg MEAM treated	8025.00±3646.34[a]	48.75±8.02[a]	15.90±2.41[a]	100.75±14.91[a]

Means with the same letter in same row are not significantly different ($p<0.05$). Values are expressed as mean±SEM for 5 rats in each group. TWBCC, Total white blood cell count; PCV, Packed cell volume; Hb, Haemoglobin.

Discussion

Toxicity studies are carried out to evaluate the adverse effects of a test substance after prolonged use and to provide information about the potential health hazards that may arise from repeated exposure over a relatively short period, such as information about target organs, the possibility of cumulative effects, and an estimate of the dose at which no adverse effect is observed. . Findings from this study showed that MEAM was relatively safe, as oral administration of 1000mg/kg and 2000mg/kg body weights of MEAM did not cause toxicity in Wistar rat.

The liver and kidneys are sensitive organs whose activities are known to be influenced by a variety of factors, including medicines and phytochemicals derived from plants, which might result in hepatic or renal failure (Chang *et al.*, 2012). Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) have been known as the marker of injury to hepatic cells (Yang *et al.* 2014). The levels of ALT, AST, and creatinine were measured to see if MEAM had caused any damage to the liver or kidney. The results of our research reveal that MEAM has no significant effect on the levels of AST and ALT, which are regarded as sensitive indicators of hepatocellular damage and can provide a quantitative assessment of the degree of liver damage when kept within limits (Olufunsho *et al.*, 2014). This corroborates the finding of Hauwa *et al.* (2017), who found no differences in the serum levels of ALP, ALT, and AST in Wistar rats administered *Annona muricata* leaf extract. Furthermore, the results of our research reveal that MEAM does not affect creatinine levels. This is consistent with Syahida *et al.* (2012) finding. The non-significant difference in the levels of these biochemical parameters indicates that the dosages of MEAM (1000mg/kg and 2000mg/kg body weight) used for this study may not interfere significantly with the metabolism of these biochemical parameters. This supports an earlier report by Unuofin and Otunola (2018) who studied acute and sub-acute toxicity of aqueous extract of the tuber of Kedrostis Africana (L.) in Wistar rats. As a result, it is acceptable to conclude that MEAM did not cause any biochemical damage to the liver or kidney. However, when comparing the 2000mg/kg MEAM treated group to the control group, there was a significant increase ($P< 0.05$) in the level of AST, but the values were far below the normal reference values, as reported by

Agbai *et al*. (2014) and Hauwa *et al.* (2017), suggesting possible hepatoprotective potential that is worth investigating.

Blood parameter analysis is important for assessing the risk of hematological system changes in humans. From our findings, MEAM appeared to increase WBC count in a dose-dependent manner, although, the increase in the two doses compared to controls was not significant. The effect of MEAM on the total WBC count could be due to the anti-inflammatory property of Annona muricata (Ana *et al*., 2018). MEAM treatment at 1000mg/kg and 2000mg/kg body weight had no significant effect on the hematological parameters of treated rats. This is demonstrated by the fact that there was no significant change in the levels of TWBCC, PCV, or Hb, demonstrating that MEAM has a non-toxic effect. Although Okoye and Efiong (2016) found a significant increase in PCV and Hb in Wistar rats given Annona muricata stem bark extract compared to the control, our findings are supported by two different authors who found no significant difference in those hematological parameters (Syahida *et al.*, 2012; Mariam *et al.*, 2021). The discrepancy between our findings and those of Okoye and Effiong (2016) could be due to differences in dosage, administration duration and time, and animal weights used in the studies.

In conclusion, this study has provided evidence of unaltered hematological and biochemical indices following the administration of methanol stem bark extract of *Annona muricata*, hence, it is non-toxic and safe in Wistar rats when given in a graded dose.

Conflict of Interest

The authors declare that there is no conflict of interest

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References

Agbai, E.O., Mounmbegna P. E., Nwafor A. (2014).The Beneficial Effects of Methanol Extract of *Annona Muricata* Seeds on Acute Hemolytic Anemia in Albino Wistar Rats. Journal of Medical Science and Clinical Research, 2(5): 958-967

Ana V. Coria-Te´llez, Efigenia Montalvo-Go´nzalez, Elhadi M. Yahia, Eva N. Obledo-Va´zquez. (2018). *Annona muricata*: A comprehensive review on its traditional medicinal uses, phytochemicals, pharmacological activities, mechanisms of action and toxicity. Arabian Journal of Chemistry, 11, 662–691

Badrie, N., Schauss, A.G. (2009). Soursop (*Annona muricata* L.): composition, nutritional value, medicinal uses, and toxicology. In: Watson, R.R., Preedy, V.R (eds.), Bioactive Foods in Promoting Health. Oxford, pp. 621–643.

Bain, B. J., Lewis, S.M., Bates, I. (2006). Basic hematological techniques in Dacie and Lewis practical hematology. 10th edition, Churchill Livingstone, Philadelphia. 25-58

Billon, H. (1869). Histores de plantes. Libraire de L. Hachette, Paris, pp. 275-276.

Blass, K.G., Thirbert, R. J., and Lam, L.K. (1974). Modified Jaffe procedure for serum creatinine. Clinical Chemistry and Clinical Biochemistry, 12:18-21

Boukandou, M.M., Mewono, L., Aboughe, A.S. (2015). Toxicity studies of medicinal plants used in sub-saharan Africa. Journal of Ethnopharmacology, 174, 618-627. 10.1016/j.jep.2015.06.005

Chang, C. J., Tzeng, T.F., Liou, S.S., Chang, Y.S., Liu, I.M. (2012). Acute and 28-day

subchronic oral toxicity of an ethanol extract of *Zingiber zerumbet* (L.) Smith in rodents. Journal of Evidence-Based Complimentary and Alternative Medicine, 2012:608284.

Correa-Gordillo, J., Ortiz, J., Sa´nchez-Mejı´a, M., Pacho´n, H. (2012). Actividad antioxidante enguana´bana (Annona muricata L.) una revisio´n bibliogra´fica. Bol. Latinoam. Caribe Plant. Med. Aromat. 11, 111–126.

Ezuruike, U. F., Prieto, J.M. (2014). The use of plants in the traditional management of diabetes in Nigeria. Pharmacological and toxicological consideration. Journal of Ethnopharmacology, 155(2), 857-924

George, V.C., Kumar, D.R., Suresh, P.K., Kumar, R.A. (2014). Antioxidant, DNA protective efficacy and HPLC analysis of *Annona muricata* (soursop) extracts. Journal of Food Science and Technology, 52 (4), 2328–2335

Hadju, Z., Hohmann, J. (2012). An ethnopharmacological survey of the traditional medicine utilized in the community of Porvenir, Bajo Paraguay India Reservation, Bolivia. Ethnopharmacology, 139 (3), 838-857

Hauwa, B. S., Gabi B., Sadiya M.A. (2017). Acute and sub-chronic profile of *Annona muricata* on Wistar albino rats. Bayero Journal of Pure and Applied Sciences. 10(2): 57-63

Monigatti, M., Bussmann, R. W., Weckerie, C.S. (2013). Ethnopharmacol. 145, 450-464

Nawwar, M., Ayoub, N., Hussein, S., Hashim, A., El-Sharawy, R., Wende, K., Harms, M., Lindequist, U. (2012). A flavonol Trigly coside and investigation of the antioxidante and cell stimulating activities of *Annona muricata* Linn. Archives of Pharmaceutical Research, 35, 761–767.

Okoye, J., Effiong, G. (2016). Hematopoetic and enzyme modulatory effects of Aqueous stem bark of *Annona muricata*. Journal of Basic and Applied Research, 2(2): 102-108.

Olufunsho A., Ismail O. I., Victor O. I., Abidemi J. A. and Alade A. (2014). Toxicological evaluation of the lyophilized fruit juice extract of *Annona muricata* Linn. (Annonaceae) in rodents. Journal of Basic and Clinical Physiology and Pharmacology, 25(4): 411–421. DOI 10.1515/jbcpp-2013-0085

Reitman, S. and Frankel, S.A. (1975). Colorimetric method for the determination of transaminases in serum. American Journal of Clinical Pathology, 28:56-63

Research, I. for L.A., (1996). National Research Council: Guide for the Care and Use of Laboratory Animals. National Academy Press Washington DC.

Schultz, F., Anywar, G., Wack, B., Quave, C.L., Garbe L.A. (2020). Ethnobotanical study of selected medicinal plants traditionally used in the rural greater Mpigi region of Uganda. Journal of Ethnopharmacology, 256, 112742.10.1016/j.jep.2020.112742

Syahida, M., Maskat, M. Y., Suri, R., Mamot, S. and Hadijah, H. (2012). Soursop (*Anona muricata* L.): Blood hematology and serum biochemistry of Sprague-Dawley rats. International Food Research Journal, 19 (3): 955-959

Tisott, G., Molin, D., Colet, C.D.F. (2013). The use of medicinal plants and herbal medicines for patients in chemotherapy in an oncology center of Ijuı´/RS. O Mundo da Sau´de 39 (3), 287–298.

UnuofIn, J.O., Otunola, G.A., (2018). Acute and subacute toxicity of aqueous extract of the tuber of Kedrostis africana (L.) on Cognition in Wistar rats. Journal of Complement and Integrative Medicine, 3(5):17–24

WHO (World Health Organization). (2005). Preventing chronic diseases a vital investment. <http://www.who.int/chp/chronic_disease_report/full_report.pdf> (accessed on 25.08.15)

Yahaya, G., Fred W., Hany A.E. (2017). *Annona muricata*: Is the natural therapy to most disease conditions including cancer growing in our backyard? A systematic review of its research history and future prospects. Asian Pacific Journal of Tropical Medicine, 10(9): 835–848

Yang, X., Schnackenberg, L.K., Shi, Q., Salminen, W.F. (2014). Hepatic toxicity biomarkers. Biomakers in Toxicology, pp 241–259