



Assessing the Impact of *Annona muricata* Aqueous Extract on Liver Enzymes in Wister Rats Exposed to Paracetamol Over Dose

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study examines the effect of the aqueous leaf extract of *Annona Muricata* on liver enzymes in wister rats using the spectrophotometric method.

Methodology: 20 Wister rats were divided into groups A, B, C, and D, of 5 rats each, housed, fed, and the experimental groups administered treatments orally in distilled water (10 ml/kg) by means of orogastric cannula for 14 days. 500g of the plant powder was submerged for 72 hours 1500ml of distilled water. The sample was taken with a rotating evaporator, an electrical evaporator extraction apparatus. With water heated to 45°C, the solvent was removed. Before use, a paste-like sample

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was made and kept in a refrigerator at 4 degrees Celsius. The animals in group A were used as the control. The animals in group B were given feeds + 20mg of paracetamol, and the animals in group C were administered 100mg of paracetamol + silymarin. In comparison, the ones in group D were given 150mg of paracetamol + *Annona muricata* extracts. The animals were then sacrificed 48 hours following the final dose. A blood sample was collected, allowed to coagulate, and then the serum was separated, while the liver was excised and utilized for biochemical analyses.

Results: The alanine aminotransferase activities (ul) recorded 9.0 ± 2.71 , 14.67 ± 1.5 , 13.50 ± 0.58 , 15.75 ± 2.50 for animals in groups A, B, C, and D respectively with corresponding activities of aspartate aminotransferase (ul) of 7.25 ± 0.50 , 27.33 ± 4.04 , 20.00 ± 2.71 , 17.25 ± 1.71 . Results revealed a significant difference ($p < 0.05$) in the activities of ALT and AST of all the treated groups. Thus, the aqueous leaf extract of *Annona muricata* could be hepato-protective. The silymarin could also be hepatoprotective, but it is less effective than *Annona muricata*. The untreated group, B, showed elevated levels of alanine aminotransferase and aspartate aminotransferase, confirming paracetamol's hepatotoxic activity.

Conclusion: The ethanol leaf extract of *Annona muricata* decreased the activities of liver enzymes in male wister rats, suggesting its hepato-protective tendency. It also does not encourage body weight loss.

Keywords: *Annona muricata*; wister rats; paracetamol; alanine aminotransferase.

1. INTRODUCTION

“Soursop (*Annona muricata*) is an evergreen tree native to Mexico, Cuba, Central America, the Caribbean, and northern South America, primarily Colombia, Brazil, Peru, and Puerto Rico. It is in some parts of Africa, especially in Eastern Nigeria, Southeast Asia, and the Pacific” [1]. “*Annona muricata* has medicinal uses, such as lowering elevated blood pressure” [2]. “Generally, the fruit and fruit juice can be taken for worms and parasites, to cool fevers, to increase the mother's milk after childbirth, and as an astringent for diarrhea and dysentery. The crushed seeds are used against internal and external parasites, head lice, and worms. The barks, leaves, and roots are considered sedative, antispasmodic, hypotensive, and nervine, and a tea made for various disorders towards those effects” [3].

“The liver is necessary for survival; there is currently no way to compensate for the absence of liver function in the long term, although new liver dialysis techniques can be used in the short term” [4]. “The highly specialized tissues in the liver regulate a wide variety of high-volume biochemical reactions, including the synthesis and breakdown of small and complex molecules, many of which are necessary for normal vital functions” [5]. “The important mechanisms of cell injury are metabolic activation of paracetamol, glutathione depletion, alkylation of proteins, especially mitochondrial proteins, and the formation of reactive oxygen/nitrogen species” [6].

“The most common liver enzymes are Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), and Alkaline Phosphatase (ALP), which are useful biomarkers of liver injury in a patient with some degree of intact liver function” [7].

“Acetaminophen (APAP, Paracetamol) is one of the most popular drugs in the world, which was first introduced and used in 1893 by Von Mering to treat pain and fever” [8]. “The effect of the drug starts about 12 minutes after its oral administration. Due to high drug administration, poisoning with this medicine is one of the most common cases of poisoning in the world, starting with symptoms such as malaise, vomiting, diarrhea, and sometimes shock. Also, excessive consumption can lead to liver necrosis and jaundice in animals and humans. In some cases, it has been observed to cause simultaneous myocardial and renal damage” [9,10,11].

2. MATERIALS AND METHODS

2.1 Collection, Identification and Laboratory Analysis of Edible Plant

Fresh leaves of *Annona muricata* were collected from the wide-growing habitat within Ughelli of Ughelli North LGA of Delta State in March 2019, and the taxonomic identity of the plant was confirmed at the Department of Plant Biology and Biotechnology, Faculty of Life Science, Delta State University, Abraka, Nigeria. “The leaves were removed from the stalk and air dried at

room temperature ($28\pm 2^{\circ}\text{C}$) to a constant weight, after which it was grounded with the sterilized machine and, sieved to fine powder and made into extracts used for the experiment" [12].

2.2 Preparation of the Extracts

Five hundred grams (500g) of the plant powder was soaked in 1500ml distilled water for 72 hours. "The extract was obtained using an electrical evaporator extraction apparatus (rotary evaporator). The solvent was extracted at a temperature of 45°C water" [13]. The paste-like extract was obtained and stored in the refrigerator at 4°C before use.

2.3 Experimental Setup

"Male wister rats obtained from the Animal House Unit of Emma-maria Scientific Research Laboratories & Consultancy, Abraka, weighing between 110-150 g, were used for this present study. Rats were housed in a ventilated animal unit provided in the Animal House Unit of the Department of Biochemistry, Faculty of Science, Delta State University, Abraka ($28 \pm 2^{\circ}\text{C}$, relative humidity 60-70 %, 12hr light/ dark cycle)". During the entire period of study the animals were supplied with standard grower mash diet (Composition of the grower's mash: Protein - 19.0% Fat -2.85% Fibre -6.00% Calcium -1.00% Available phosphate -0.45% Energy -2875 KGC (Animal Care Services Konsult (NIG) LTD), Asaba, Delta State) and water *ad libitum*, in a standard wire meshed wooden cages for 7 to 10 days prior to commencement of the experiment. In this study, all the animal experimentation was carried out according to the guidelines of the Institutional Animal Ethics Committee (IAEC).

Wister rats were divided into four (A to D). Group A, which was the normal control (positive control), was fed with regular feed and water daily for 14 days; Group B, which was the hepatotoxic control, received Paracetamol (2.0 g/kg body weight, p.o.). Group C rats were given Paracetamol + extracts of *A. moricata* orally (150 mg/kg), while Group D rats were given Paracetamol + Silymarin (100 mg/kg). All the treatments were given orally in distilled water (10 ml/kg) using an orogastric cannula for 14 days. Paracetamol (2.0 g/kg body weight of animal) suspension was prepared by using 0.5% Tween 80 and subjected to hepatoprotective activity against paracetamol-induced liver damage. Animals were sacrificed 48 hours after the last injection. Blood was taken, allowed to clot, and

serum was separated. The liver was dissected and used for biochemical studies.

At the end of the treatment period, the animals were sacrificed 24 hours following the last given dose and the time of sacrifice, the weight was taken, and then cervical decapitation was conducted before a laparotomy section was carried out. Blood samples were collected from the vena cava and heart and placed into sample bottles. The tubes were then centrifuged at 4000rpm for 10 minutes to obtain and stored at -8°C pending biochemical analysis using spectrophotometer methods with reagent kits.

2.4 Laboratory Analysis

In the laboratory, the estimation of alanine aminotransferase (ALT) activity in the serum and tissue homogenates and aspartate aminotransferase (AST) activity in the plasma and tissue homogenates was carried out by the method of Reitman and Frankel [14].

The assay for ALT is based on the enzyme's reaction.

Alanine + α -ketoglutaric acid \rightarrow pyruvic acid + glutaric acid, and L-Aspartic acid + α -ketoglutaric acid \rightarrow Oxaloacetic acid + L-glutamic acid, respectively. The activity of the enzymes was then extrapolated from a standard curve and expressed as unit/ml.

2.5 Data Analysis

All the data are expressed as mean \pm standard deviation of mean SD. Statistical comparisons were performed using ANOVA, followed by Fisher's least significant difference (LSD). A p-value of less than 0.05 ($p < 0.05$) was considered significant.

3. RESULTS

3.1 Alanine Aminotransferase

The mean ALT level for the test group rats was found to be higher when compared with the normal control group rats NC. All test groups were significantly different ($p < 0.05$) from the normal control group.

3.2 Aspartate Aminotransferase

The mean AST level for the test group rats was found to be higher when compared with the normal control group rats NC. All test groups were significantly different ($p < 0.05$) from the normal control group.

Table 1. The initial and final weights of the rats used for the experiment.

Groups	Experimental		
	Initial weight (gm)	Final weight (gm)	Difference in weight (gm)
A	123.38±1.67	122.25±0.79	0.85±0.53
B	117.83±1.17	107.25±1.07	-10.52±2.02
C	124.05±1.07	106.08±1.13	17.73±1.46
D	127.71±2.49	122.55±1.54	-3.64±1.19

Table 2. Serum Aminotransferases

Groups	Experimental	
	ALT (mg/dl)	AST (mg/dl)
A	9.00±1.21a	7.25±0.22a
B	14.67±0.51b	27.33±1.81b
C	13.50±0.26c	20.00±1.21c
D	15.75±1.12d	17.25±0.76d

Values are expressed as MEAN±SEM. Value with different alphabet superscripts in the same row indicates a significant difference (p<0.05).

Keys ; A =Normal Control B=20g of paracetamol C=100mg paracetamol + silymarin D =150mg paracetamol + Annona Muricata

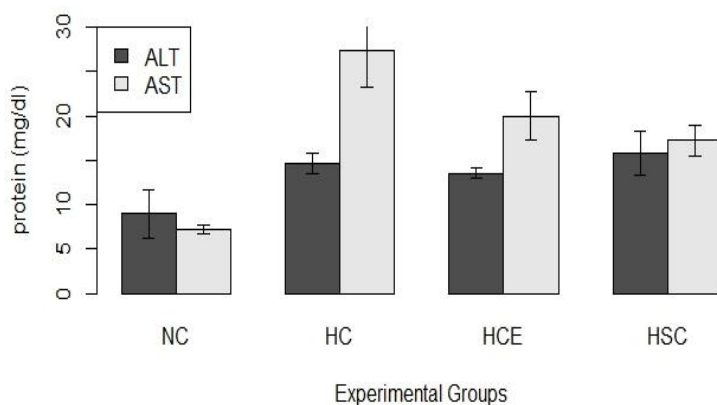


Fig. 1. Effect of administration of plant extracts on serum aminotransferases of paracetamol overdosed wister rats

NC: Normal control group

HC: Hepatotoxic control group

HCE: Animals given Paracetamol + Extracts of *A. muricata*

HSC: Animals given Paracetamol + Silymarin

4. DISCUSSION

From the study, Group C (the group treated with *Annona muricata*) shows low levels when compared with the other treatment groups but is still significantly high when compared to the normal control group. The mean alanine level of group B was shown to be higher than that of the normal control group, with the aspartine level ridiculously higher than that of the standard

group. Group D also showed a high level of the enzymes, but it was not as high as group B. The increased serum alanine and aspartate aminotransferase levels in rats are in line with previous works carried out [14-16]. This implies that although *Annona Muricata* could be used to treat paracetamol overdosed hepatotoxicity, it is dose-dependent, and the wrong administration at either a higher or lower dose could contribute to the very problem we are trying to solve. Silymarin

could also be used to treat paracetamol toxicity, but it is not as effective as *Annona muricata* leaf extract.

“Aqueous leaf extract of *Annona muricata* significantly reduced the activities of alanine aminotransferase in the rats. It was recorded that the treatment of wister rats with *Annona muricata* aqueous leaf extracts significantly reduced the elevated activities of the alanine aminotransferase” [17]. “There was a significant decrease in the activities of AST in the wister rats administered with the extracts. *Annona muricata* leaf extracts significantly reduced the elevated serum levels of AST” [18,19].

5. CONCLUSION

In conclusion, the ethanol leaf extract of *Annona muricata* decreased the activities of liver enzymes in wister rats, which suggests its hepato-protective tendency. It also does not encourage body weight loss.

ETHICAL APPROVAL

This research design was reviewed and approved by Department of Biochemistry Technology, Science Laboratory Technology, Faculty of Sciences, Delta State University.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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