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Ethanolic extract of *Annona muricata* leaf and its effect on the liver

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ABSTRACT

The effect of ethanolic extract of *Annona muricat*a leaf on the liver of albino rats were investigated in this study. Twenty (20) male albino rats weighing between 180-200 g were used in this study. The animals were divided into groups A, B, C and D. Group A was treated with 2 ml/kg body weight of distilled water, Group B was treated with 100 mg/kg body weight of the extract, Group C was treated with 200 mg/kg body weight of the extract and Group D was treated with 300 mg/kg body weight of the extract. The treatments were given orally and lasted for a period of 30 days. After the last day of treatment, the animals were sacrificed and the liver harvested, weighed and fixed in 10% formal saline for histological studies. Blood samples were collected through cardiac puncture for biochemical analysis. Data were analysed using one-way ANOVA and SPSS version 2.0. Results showed a significant ($P<0.05$) increase in liver weight when compared to control and a significant ($P<0.05$) increase in serum levels of ALP, ALT and AST at highest dose. Histopathological findings showed distortions of the liver cytoarchitecture with the highest dose having a more significant effect. Consumption of ethanolic extract of *A. muricata* leaf at higher doses has the potential of causing liver damage. Thus, its consumption should be regulated or better still taken at lower doses.

Keywords: *Annona muricata*; Liver; Liver enzymes; Albino rats

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INTRODUCTION

Herbal plants have been used throughout human history and have been considered relatively safer with its increase in demand compared to conventional drugs[1]. Theses herbal medicines are effective as conventional medicines and also have the potential to cause harmful side effects[2]. The toxic effects of these medicinal plants are sometimes not taken into consideration.

Annona muricarta (soursop) is an evergreen, terrestial tree native to Brazil, Central America Colombia, Cuba, South America, South Asia and Africa. It belongs to the member of *Annonaceae* family. The plant have been reported to have several beneficial effects with its leaf, bark, root and stem extract shown to have antibacterial effect[3,4]. The leaves of *A. muricata* are used as diuretic, depurative, in the treatment of blisters, edema, hygroma, brucellosis, dropsy and undulating fever[5]. Further research revealed its anti-malarial [6], antimutagenic[7] and anti-fungal properties[8].

A. muricata have been known to possess bioactive substances known as annonaceous acetogenins. These acetogenins have cytotoxic properties that can inhibit the mitochondrial complex I enzyme and inhibition of this enzyme significantly reduces the production of energy (ATP) and causes cancer cell death[9,10]. This inhibitory effect could occur in normal cells, particularly those with high ATP requirements, such as the brain, liver, and kidney.

The liver is a vital organ in the body with a wide range of metabolic functions. It is very important for survival and cannot be compensated for in the absence long term liver function; however, dialysis can be done in short term[11]. The performance of the liver is highly dependent on the availability of ATP in the cells. A decrease in ATP production may result in degeneration of cells or apoptosis[12,13].

MATERIALS AND METHODS

3.1 Breeding of Animals

Twenty (20) male Wistar rats weighing between 180-200g were procured from the Animal House of the Department of

Anatomy, Nnamdi Azikiwe University. The rats were kept in standard cages under normal temperature (27-30°C), with each cage having wire gauze for cross ventilation. The ethical committee of the College for animal care and use approved the study design in compliance with the National regulation for animal research. The animals were acclimatized for a period of two weeks before commencement of treatment. They were fed with normal rat chow and water *ad libitium*.

Preparation of the extract

Fresh leaves of *A. muricata* were procured from Uturu, Abia State. They were identified at the herbarium units of the Department of Botany, Nnamdi Azikiwe University Awka, Anambra State. The leaves were washed in a basin of water to remove dirt and dried under ambient temperature. The dried leaves were ground using laboratory blender to a coarse powdery form. 600g of the powder was macerated in four (4) litres of ethanol, sealed and allow for 48hrs. After 48 hours, the mixture was sieved using a porcelain cloth and was further filtered using filter paper into a clean glass beaker. The filtrate was later dried using rotary evaporator into a jelly-like/paste-like form and stored in refrigerator for future use.

Experimental Design

The twenty (20) rats were weighed and randomly allocated into four (4) groups of five (5) animals. The groups were designated as a group A, B, C, and D. Group A served as the control group and was administered 2 ml/kg body weight of distilled water. The experimental groups B, C, and D were administered with 100 mg/kg, 200 mg/kg and 300 mg/kg body weight of the extract of *A. muricata* respectively.

The administration was given orally between the hours of 10-11am daily and lasted for thirty (30) days. Twenty four (24) hours after the last dose, the animals were anaesthetized by chloroform inhalation and dissected. The liver tissues were harvested and fixed in 10% formal saline for histological examination.

Histopathological Examination

The liver tissue was processed by passing them through histochemical methods of fixation, dehydration, clearing, impregnation,, embedding, sectioning, mounting and staining. Fixation was carried out in 10% formal saline and dehydration was carried out in ascending grades of alcohol (50%, 70%, 80%, 95% and absolute alcohol) and then cleared in xylene after which embedding in paraffin wax was carried out. Sections of about 3-5µm was obtained using a rotatory microtome. The sections were later deparaffinised, hydrated and stained using haematoxylin and eosin (H&E) dye. They were later mounted using neutral dibutylphthalate xylene (DPX) medium for microscopic examination at x150 magnification.

Statistical Analysis

Data from liver weight and serum enzyme biomarkers were subjected to the Analysis of Variance (ANOVA) test followed by multiple comparisons using Least Significant Difference (LSD). The levels of significance were considered at $P<0.05$ and data was expressed as Mean ± SEM.

RESULTS

Table 1: Effect of ethanolic extract of *A. muricata* leaf on the Liver weight

GROUPS	LIVER WEIGHTS
A	2.76±0.06
B	3.81±0.12**
C	3.05±0.04
D	3.09±0.20

**P<0.001 when compared to the control

Table 2: Effect of ethanolic extract of *A. muricata* leaf on the Liver enzymes

		Mean±SEM	P-Value	F-Value
Alanine Transaminase (U/L)	Group A	40.33±3.92		
	Group B	30.00±0.57	0.034	77.224
	Group C	22.66±4.05	0.002*	
	Group D	79.00±0.57	0.000**	
Aspartate Transaminase (IU/L)	Group A	26.66±0.88		
	Group B	23.00±2.88	0.494	19.483
	Group C	23.00±6.24	0.494	
	Group D	56.00±2.08	0.000**	
Alkaline Phosphatase (IU/L)	Group A	56.00 ±0.57		
	Group B	65.00 ±5.13	0.378	256.074
	Group C	51.00 ±12.28	0.618	
	Group D	75.33 ±2.90	0.000**	

Histopathological findings

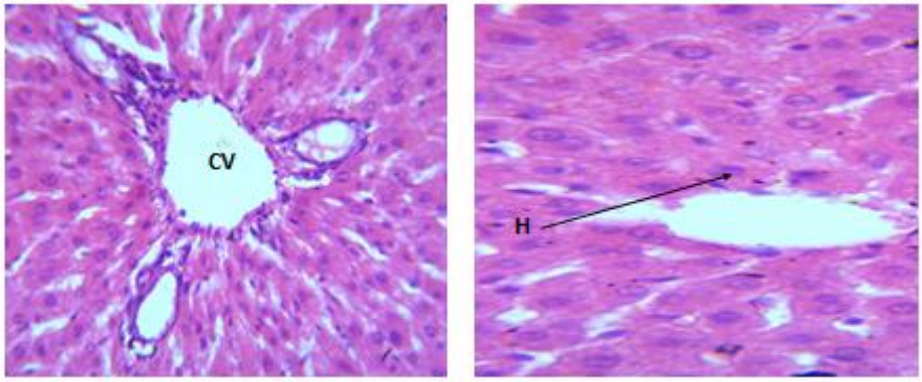

Figure 1: Photomicrograph section of group A animals administered 2 ml/kg body weight of distilled water showing normal hepatic architecture with central vein (CV), hepatocyte (H), portal triad and well perfused cytoplasm.

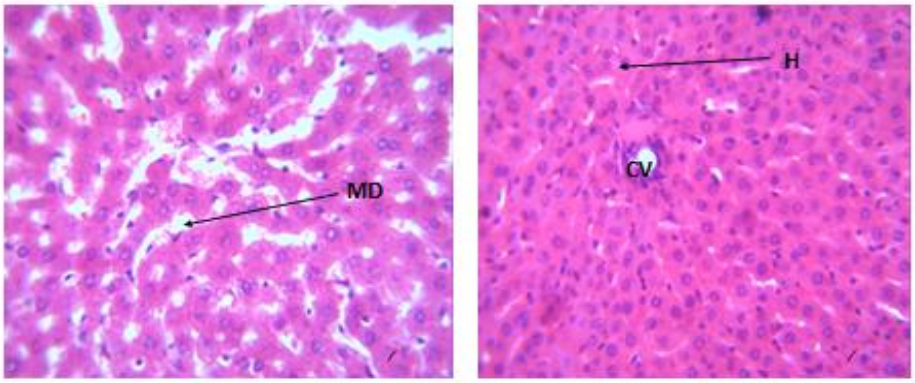

Figure 2: Photomicrograph section of group B animals administered 100 mg/kg body weight of *A.muricata* showing mild distortion (MD) with normal central vein (CV), hepatocyte (H) and well perfused cytoplasm.

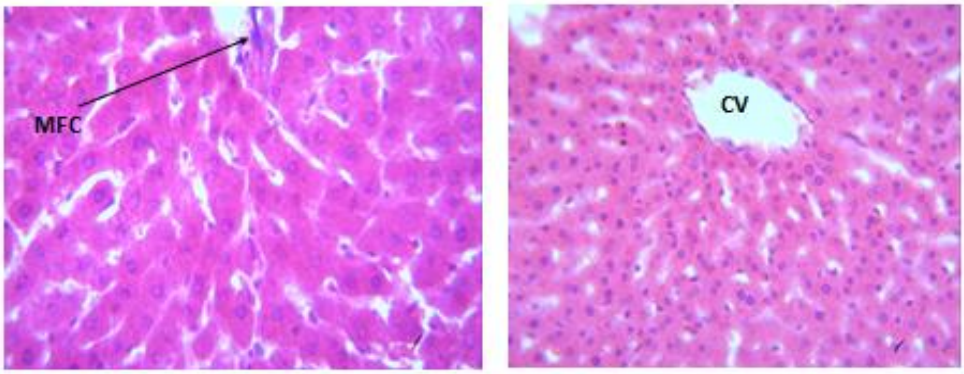

Figure 3: Photomicrograph section of group C animals administered 200 mg/kg body weight of *A.muricata* showing well perfused hepatic tissue that appears normal with mild fatty change (MFC)

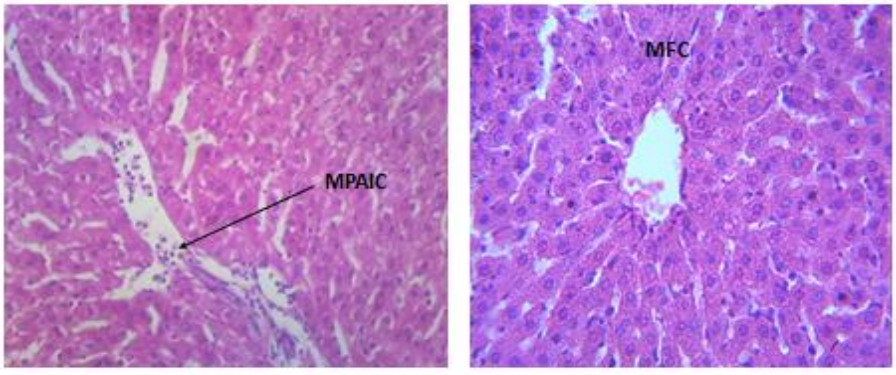

Figure 4: Photomicrograph section of group D animals administered 300 mg/kg body weight of *A.muricata* showing moderate portal aggregate of inflammatory cells (MPAIC), dilation of the sinusoid and moderate fatty (MFC).

DISCUSSION

Medicinal plants have shown promising results in the treatment of certain diseases with some of them showing toxic effect to health. Hepatotoxicity being associated with liver damage serves as a diagnostic tool in determining liver disorder. The activities of enzymes ALT, AST and ALP in serum are also used routinely to assess the functional status of the liver both in clinical and experimental settings with an elevated level of these enzymes indicating liver dysfunction when compared to control.

The results of this study revealed several cytotoxic architectural changes in the liver of the treated rats. The toxic effect of *A. muricata* leaf extract may be due to the presence of acetogenins in the leaves that causes liver toxicity via increasing calcium concentration[14].

There was a significant increase (P<0.05) in liver weight of the animals in group B when compared to the control. However, there was no significant difference in the weights of the liver of animals in group C and D when compared with the control. This increase in liver weight could be pathological in the sense that the liver might have been inflamed due to toxicity.

The extract of A. muricata had no significant effect on ALP and AST activity at 100mg/kg and 200mg/kg but there was a significant (P<0.05) increase in the serum levels of ALP in the group administered with 300mg/kg body weight indicating a possible liver damage. This contradicts the work of Nwogu *et al.*[15] who reported a significant reduction in the elevated serum levels of ALP in acute liver damage induced by different hepato-toxins.

Ethanolic extract of *A. muricata* leaf significantly (P<0.05) increased the activities of ALT at the highest dose. This is not in support with reports by Owolabi *et al.*[16], who reported a significant reduction in the elevated activities of the ALT.

Histopathological results revealed distortions of the liver cytoarchitecture with the highest dose having a more significant effect. This correlates with that of the biochemical parameters. These distortions could be due to the presence of acetogenins in the extract which have been reported to inhibit the mitochondrial complex I enzyme. Inhibition of this enzyme significantly reduces the production of energy (ATP) and causes cell death[9,10]. With the performance of the liver being dependent on the availability of ATP in the cells, its reduction may result in degeneration of cells or apoptosis[12,13]. This is in agreement with the work of Ezejindu *et al.*[17].

CONCLUSION

The findings from this research indicate that the risk of consuming this leaf is closely related to the doses administered; with higher dosage resulting in hepatic damage. Therefore, its consumption should be regulated and taken at lower doses.

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None

Conflict of Interest

There was no conflict of interest during this research

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