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**Research Article**

# The Effects of Graviola Leaf Aqueous Extract on the Liver of Wistar Rats

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**Abstract:**

The objective of this study is to evaluate the histological effect of Annona muricata aqueous leaf extract on the liver of adult wistar rats. Twenty four adult wistar rats weighing 180-205g were used for the study. They were divided into four groups (A, B, C and D) of six animals each. Group A served as the control and received distilled water; the experimental group B, C and D were orally administered 0.2ml, 0.4ml and 0.6ml of Annona muricata aqueous leaf extract respectively for twenty one days. Both the control and experimental groups were weighed, and sacrificed under chloroform anaesthesia at the end of the period of administration. liver tissues were removed and fixed in 10% formaline for histological studies. The body weight result showed reduction in the groups C and D animals treated with 0.4ml and 0.6ml of Annona muricata ethanolic leaf extract when compared with the control while group B increased significantly relative with the control. Histopathological results showed that group B presented mild cellular infiltration and vascular congestion, Groups C was observed to have vascular congestion and pyknotic nuclei while Group D showed area of aggregates of cellular infilteration, vascular congestion and vacuolated nuclei. This study suggests that high doses of administration of Annona muricata aqueous leaf extract may cause adverse effects on the liver cells.

## Introduction

Annonaceae, the custard apple family is a family of flowering plants consisting of trees, shrubs, or rarely lianas (Kedari and Khan, 2014). With about 2300 to 2500 species and more than 130 genera, it belongs to the genus *Annona* and the family is concentrated in the tropics, with few species found in temperate regions (Kedari and Khan, 2014). About 900 species are Neotropical, 450 are Afrotropical, and the other species Indomalayan. Graviola tree, or soursop in English (Scientific Name: *Annona muricata Linn*.) is ethno medicinally important species from this family. Graviola is adaptable to tropical climate and are currently cultivated for its fruit in most Southeast Asian countries such as Malaysia, Indonesia and Philippines (Kedari and Khan, 2014). It is also known as *guanabana* (Spanish, El Salvador) *huanaba* (Guatemala), *zopote de viejas* (Mexico), or *cabeza de negro*(Venezuela) , *catoche* or *catuche* (Argentina), *anona de puntitas* or *anona de broquel*; (Bolivia) *sinini*; (Brazil), *araticum do grande*, *graviola*, or *jaca do Para*; (Netherlands) amongst a few. It is a small, upright tropical evergreen, low-branching and bushy but slender tree, which can reach a height of 7.5-9 m. The large evergreen leaves are smooth and glossy and have a dark green upper surface. The fruits are usually oval or heart-shaped and 10-30 cm long and up to 15 cm in width. The skin of the fruit is leathery and covered with curved, soft, pliable spines. The inside of the fruit is cream-colored and is divided into segments. Closely-packed segments are seedless and other segments have a single oval, smooth, hard black seed. One piece of large fruit can contain a dozen to 200 or more leaves (Morton, 1987).

Many indigenous plants are used without the actual knowledge of their toxic potentials (Musa *et al.*, 2005). Despite the availability of a wide range of approaches in disease management, low income developing countries with limited access to modern veterinary drugs and services, rely on herbal preparation for the health care needs of their animals

(Jung *et al.*, 2011;Offiah *et al.*, 2011). This could possibly lead to the misuse and abuse of such plants supported by the fact that most herbal concoctions are not officially regulated (Riddle, 1992;Onyeyili, 2000).

Available evidence suggests that approximately 80% of Africans rely on traditional healthcare practitioners and medicinal plants for their daily healthcare needs (Johnson *et al.*, 2007; McKay *et al.*, 2007); therefore the need to carry out this study on the effect of Gaviola leaf on the liver considering the facts that hepatic dysfunction is one of the leading cause of death worldwide (Vanhecke *et al*., 2006; Mendis *et al*., 2011).

However, there is limited information available on the effect of Graviola leaf on the liver of Adult wistar rats. Therefore, this present study is set to investigate the effect of aqueous extract of Graviola leaf on the liver of adult Wistar rats.

It is a known fact that graviola plants possess nutritional and therapeutic attributes; however, extensive study has not been done on its effect on the liver. This research will make a valuable contribution to other researchers as regards the impact of graviola plants on medicinal plants, in order to more efficiently and appropriately respond to the health needs of the growing populace. Furthermore, this study will provide information on the possible effects of graviola plant consumption on Liver as well as providing the basis for its continuous usage or stoppage.

## Materials and Methods

### Geographical Description of Study Area

This study was carried out in the College of Medical Sciences, Ambrose Alli University in Edo State. Edo state lies between longitude 06º 04′E and 06º43′E and latitude 05º 44′N and 07º 34′N with a landmass of 17,450sq.km located in the South South geographical zone of Nigeria with a population of 3.1 million people (WorldGazzetter, 2007).

### Experimental Animals/Housing Condition

Twenty (24) Adult Wistar rats of comparable sizes and weights were procure from available animal house and transferred to the experimental site where they were allowed one week of acclimatization. During this period of acclimatization, the rats were fed growers' mash and water provided *ad libitum*.

### Animal Grouping

The experimental animals were separated into four groups (A – D). Group A had six rats (n = 6) while groups B-D contain six rats (n = 6) each using 4 big cages to house them. Group A served as the control, while groups B - D served as the test groups.

Group A served as the control and received only the normal feed (grower's mash) and water with no administration of Graviola leaf aqueous extract while Group B, C and D received different doses of Graviola leaf aqueous extract.

### Study Duration

The administration of Graviola leaf aqueous extract to the test animals lasted for three (3) weeks.

### Preparation of Substance

The leaves of *Annona muricata* were obtained and taxonomically identified by a botanist in the Department of Botany, Faculty of Natural Sciences, Ambrose Alli University, Ekpoma. They were sun-dried and crushed using laboratory blender. Extraction was done using distilled water. 200mg of this extract/kg body weight was dissolved in 10mls of distilled water and administered to the animals.

### Substance Administration

The doses that were employed in this study are as described by Ezejindu *et al*., (2014).

- **Group A** (Control) received of normal feed (growers' mash) and water daily for four (4) weeks.
- **Group B;** received 0.2ml of *Annona muricata* aqueous leaf extract for three (3) weeks.
- **Group C;** received 0.4ml of *Annona muricata* aqueous leaf extract for three (3) weeks.
- **Group D;** received 0.6ml of *Annona muricata* aqueous leaf extract for three (3) weeks.

### Sample Collection and Analysis

Weights were measured before and after acclimatization and similar weight measurements were done at the end of each week and the average weight recorded accordingly. The liver of each rat were obtained at the end of the administration (3 weeks) under chloroform anaesthesia and fixed in 10% formalin for histological processing.

### Histological Processing

The tissues were processed using automatic tissue processor according to the standard processing schedule. The fixed plastic cassette tissues in 10% formalin were automatically processed by passing them through different grades of alcohol as follows:

| | |
|---|---|
| 70% alcohol | 1hr |
| 80% alcohol | 1hr |
| 90% alcohol | 1hr |
| 90% alcohol | 1hr |
| 95% alcohol | 1hr 30mins |
| Absolute alcohol I | 2hrs |
| Absolute alcohol 11 | 2hrs |
| Xylene 1 | 1hr 30mins |
| Xylene II | 1hr 30mins |
| Molten paraffin wax 1 | 2hrs |
| Molten paraffin Wax II | 2hrs |

After the last timing, the tissues were removed from their plastic cassettes and placed at the centre of the metallic tissue mould and then filled with molten paraffin wax. They were also left to solidify after which they were now placed in the refrigerator at 5$^{o}$C for 15 minutes. After the blocks were cool in the refrigerator for the time stated above (15 minutes), the blocks were removed from the metallic case using a knife and after which the paraffin wax at the side of the blocks were removed.

The blocks were trimmed and cut serially at 3mm on a rotary microtome. The sections were floated in water bath at 55°C and picked up by the use of a clean frosted end slides. The frosted end slides were placed on the hot plate for 40 minutes for adequate attachment of the sections on the slides after which the sections were dewaxed, hydrated, air dried and stored in a slide box ready for staining process.

**Staining Procedure**

Sections for general tissue structure were stained by Haematoxylin and Eosin technique as follows:

1. The sections were dewaxed in 2 changes of xylene 5 minutes
2. The sections were hydrated through descending grades of alcohol (absolute, 95%, 80% and 70%).
3. The sections were stained in Harris haematoxylin 5 minutes
4. The sections were rinsed in running tap-water to remove excess stain
5. The sections were differentiated in 1% acid alcohol briefly
6. The sections were blued in running tap water 10 minutes
7. The sections were counterstained with 1% eosin 30 seconds
8. Sections were finally rinsed in water, dehydrated in ascending grades of alcohol (70%, 80, 95% and absolute)
9. The sections were cleared in xylene, air-dried and mounted with dibuthylphthalate propylene xylene (DPX).

The slides were examined under a light microscope and photomicrographs were taken.

**Data Analysis**

The obtained data were subjected to statistical analysis using SPSS (statistical package for social sciences version 17). The test groups' values were compared with the values of the control group using ANOVA (Scheffe) at 95% level of confidence.

## Results

**Results on Body Weight Changes**

Table 1 shows the body weight changes in the test groups. Although at every stage of the weight determinations, the entire groups (A, B, C and D) presented body weight gains. Body weights were similar in the control and tests groups at baseline (before acclimatization) and after acclimatization. However, variations in body weight gain were observed between the control and test rats. Comparatively, these body weight variations were significant in group D (134.13 ± 5.25g) after second week of Graviola administration, and group C (152.25 ± 2.71g) and D (151.25 ± 4.56g) after the third week of administration.

**Table 1: Body weight changes of rats fed graded doses of *Annona muricata* leaf aqueous *extract* at various interval.**

| Stages of weight measurement | Control Group A | Test groups | | |
|---|---|---|---|---|
| | | B (0.2ml/ml GL) | C (0.4ml GL) | D (0.6ml GL) |
| Weight. Before Acclimatization | 102.85 ± 4.98[a] | 101.39 ± 5.17[a] | 103.97 ± 5.33[a] | 100.68 ± 6.68[a] |
| Weight. After Acclimatization | 112.71 ± 5.09[a] | 111.25 ± 2.76[a] | 111.75 ± 4.98[a] | 113.25 ± 3.11[a] |
| Weight. After 2nd week of Gaviola administration | 140.86 ± 6.26[a] | 137.00 ± 4.99[ab] | 137.38 ± 4.41[ab] | 134.13 ± 5.25[b] |
| Weight. After 3rd week of Gaviola administration | 160.86 ±8.11[a] | 155.75 ± 6.09[ab] | 152.25 ± 2.71[b] | 151.25 ± 4.56[b] |

Values are mean ± SD, Wt= weight; GL= *Graviola leaf extract*; value in a row with different superscripts are significantly different at P <0.05.

**4.2 Histological Observation**

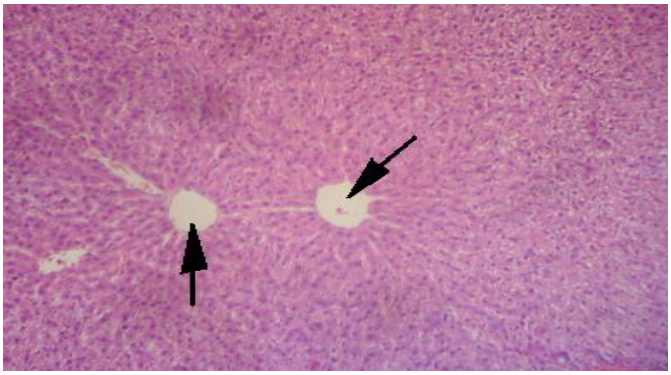

**Plate 1: Photomicrograph of control liver section (H&E x100) showing intact liver cytoarchitecture with visible central veins (arrows).**

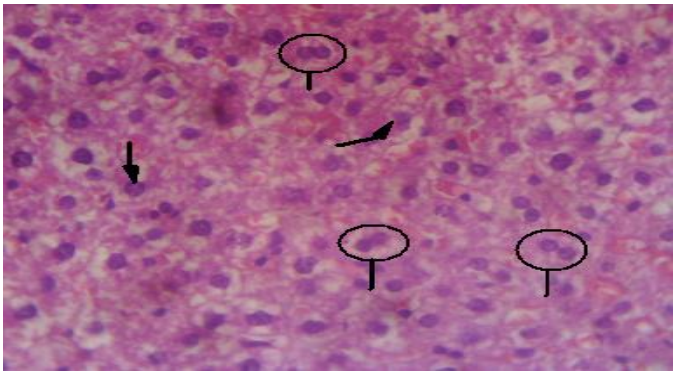

**Plate 2: Photomicrograph of control liver section (H&E x400) showing intact liver cytoarchitecture with visible hepatocytes (arrows) and some bi-nucleate liver cells (encircled).**

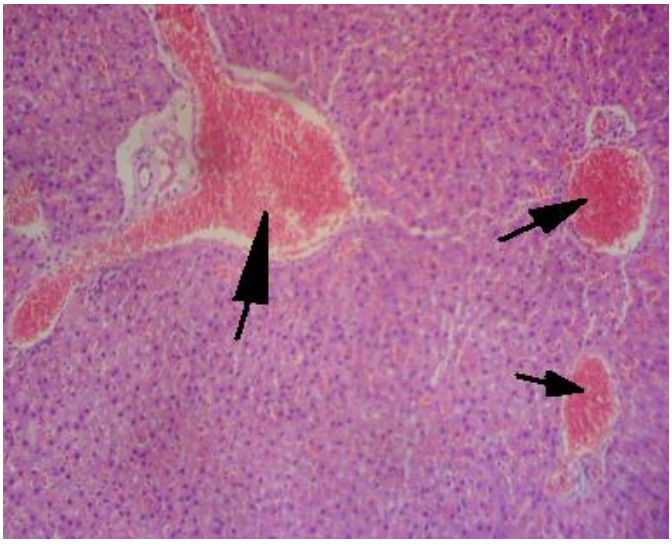

**Plate 3: Photomicrograph of group B liver section (H&E x100) showing liver cytoarchitecture with vascular congestions (arrows)**

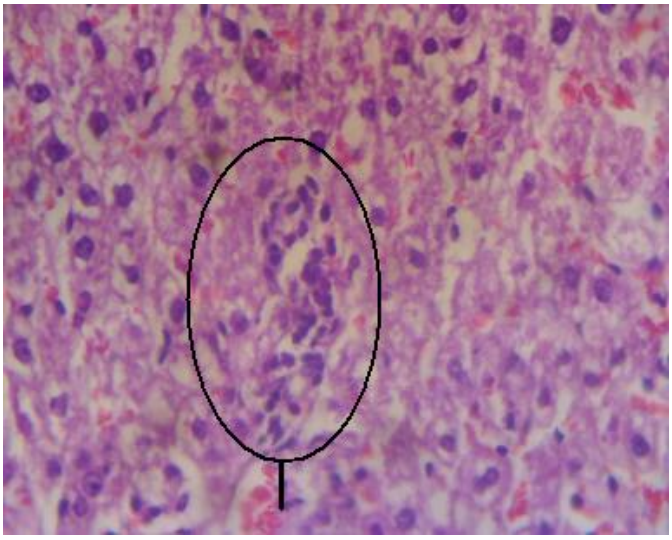

**Plate 5: Photomicrograph of group B liver section (H&E x400) showing liver cytoarchitecture with mild cellular infiltrates (encircled)**

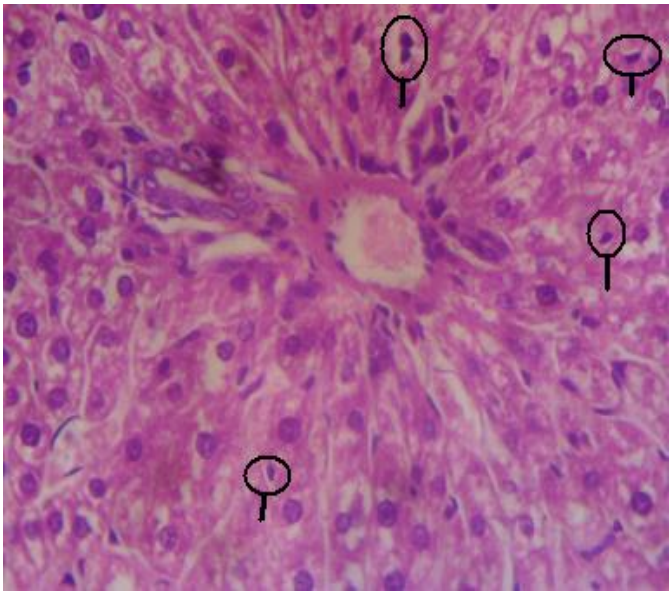

**Plate 7: Photomicrograph of group C liver section (H&E x400) showing liver cytoarchitecture with some pyknotic nuclei (as encircled)**

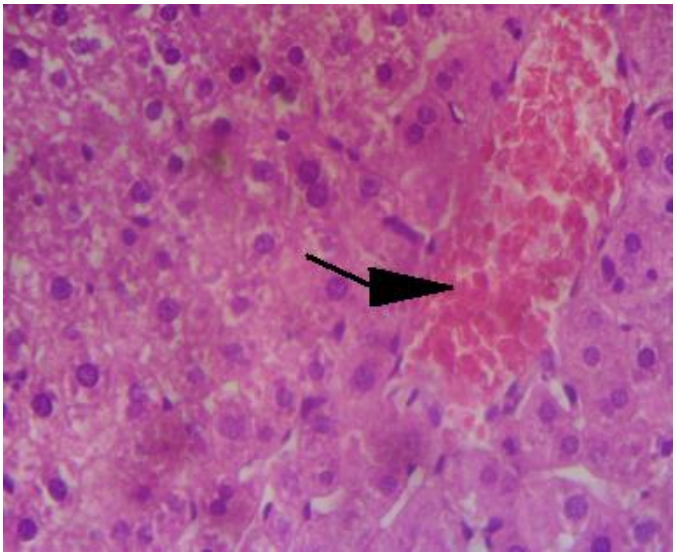

**Plate 8: Photomicrograph of group C liver section (H&E x400) showing vascular congestion (arrow)**

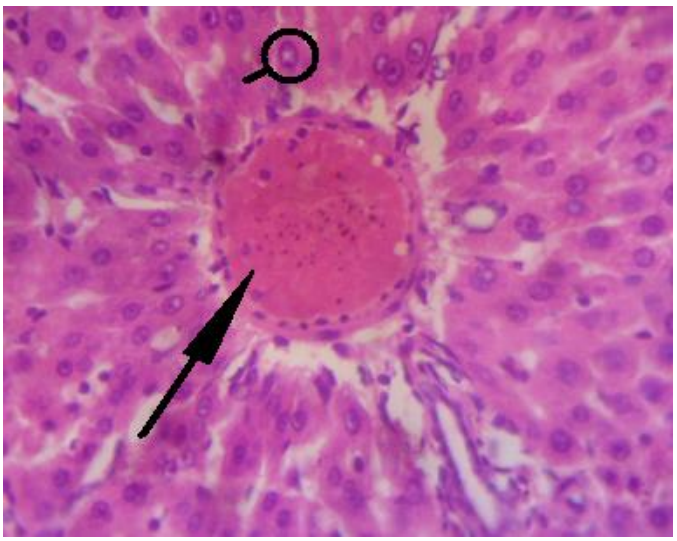

**Plate 10: Photomicrograph of group D (H&E x400) showing liver cytoarchitecture with mild vascular congestions (arrow) and vacuolated nuclei (encircled)**

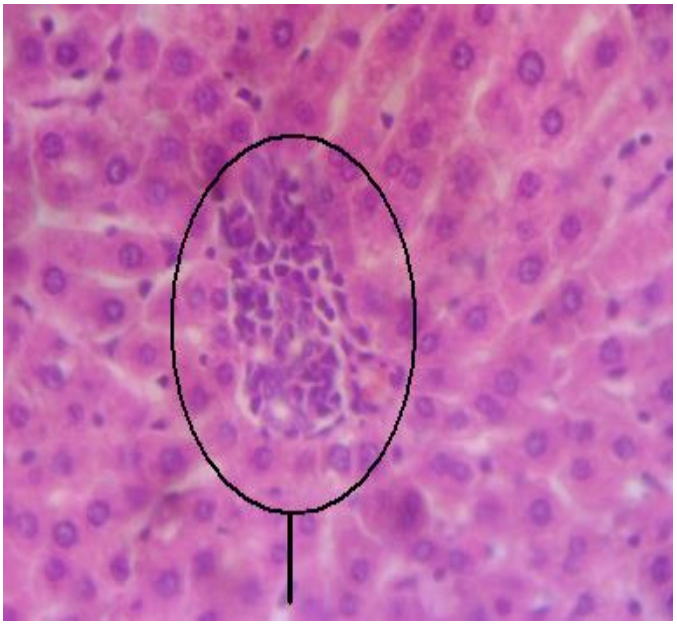

**Plate 11: Photomicrograph of group D (H&E x400) showing liver cytoarchitecture with aggregates of cellular infiltrates (encircled)**

## Discussion

*Annona muricata* leaf extract is used in the treatment of various bacterial infectious diseases such as pneumonia, diarrhea, urinary tract infection and even some skin disease (Dai *et al.,* 2011).

In the present study, *Annona muricata* aqueous leaf extract was administered to adult wistar rats to investigate whether this plant leaf extract could be toxic to the liver of adult wistar rats. A reduction in body weight of the wistar rats were observed in the groups C and D with the highest doses of aqueous leaf extract of *Annona muricata*. The reduction in weight may be due to reduced food intake which may be secondary to feeling of fullness and loss of appetite after administration of the extract. This was also reported by Ezejindu *et al.* (2014), who reported decrease in weight in test animals after administration of Annona muricate ethanolic leaf extract.

In the histopathological examinations, the extent of liver damage was assessed. The animals in group D showed hepatotoxicity, this is evidenced by the presence of vascular congestion, vacuolated nuclei and aggregates of cellular infiltration. Group C also showed area of vascular congestion and pyknotic nuclei. The group B result showed mild cellular infiltration and vascular congestion.

## Conclusion

This study suggests that oral administration of *Annona muricata* aqueous leaf extract at high doses could cause adverse histopathological changes in the liver cyto-architecture and decrease in body weight.

### Recommendation

Based on the histological observation presented in this study, it may therefore be recommended that;

1. There is need for further studies to determine its toxic dose
2. Furthermore, the use of *Annona muricata* leaf extract in herbal remedies should be regulated.
3. People who resort to the use of this plant as herbal therapy should exercise caution in the dosage taken considering the pathologic effects on the liver.

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