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# Basal Biochemical Studies of the Influence of *Annona muricata* on Some Liver Function Parameters of Treated Adult Wistar Rats

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ABSTRACT

**Background:** This study examines the effects of *Annona muricata* - commonly known as soursop on liver function parameters in adult Wistar rats. *Annona muricata* is a tropical fruit with potential hepatoprotective properties, traditionally used for various medicinal purposes. However, data on the scientific investigations regarding its impact on liver function parameters are limited, thus, it is based on this premise that this research was designed and carried out.

**Materials and Methods**: In this study, adult Wistar rats were administered extract from different morphological parts of *Annona muricata* (Leaves, Fruit, root bark, and stem bark) orally for 28 days, followed by an assessment of liver function parameters. The rats were divided into control and 5 treatment groups. The control group received a standard diet, while the treated groups received increasing doses (100 mg/kg, 200 mg/kg, 400 mg/kg, 600 mg/kg, and 800 mg/kg) of the different morphological parts *Annona muricata* plant (leaf, fruit-pulp, root-bark, and stem-bark) alongside the standard diet. The parameters include Liver plasma protein, lactate dehydrogenase (LDH), total albumin, globulin (GLB), serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), Albumin, liver tissue proteins, Gamma-Glutamyl Transferase, Plasma protein total bilirubin, conjugated and unconjugated bilirubin levels were evaluated to monitor potential hepatotoxicity or hepatoprotective effects.

**Results**: Slight alterations in the liver parameters were observed for the various morphological parts of *A. muricata,* especially for the high-dose treated groups. Also, mild histological changes were also observed in the liver tissues of the high doses treated groups. The results obtained from this study provide insights into the effects of *Annona muricata* on liver function parameters.

**Conclusion**: These findings will contribute to the existing knowledge on the hepatoprotective potential of *Annona muricata* and may have implications for its application as a therapeutic agent.

**Key words**: *Annona muricata*; liver markers; hepatoprotective; hepatotoxicity; histology; Soursop.

## INTRODUCTION

*Annona muricata*, often known as soursop or Graviola, is a fruit-bearing tree that belongs to the Annonaceae family (1), which has about 2300 species and 130 genera (2,3). The huge fruit's sour and sweet flavor gave the plant its soursop moniker (4). *Annona muricata* is native to the warmest tropical areas in South and North America and is now widely distributed throughout tropical and subtropical parts of the world, including India, Malaysia and Nigeria, Australia, Africa, (5). Its leaves, bark, and fruit have long been used in traditional medicine for various purposes (4).

Over the years, numerous scientific research studies have been conducted to investigate the potential health benefits and therapeutic properties of different parts of the *Annona muricata* plant. Several studies have explored the potential anticancer effects of *Annona muricata* (6−10). Research suggeststhat the plant contains active compounds, including annonaceous acetogenins, which demonstrate selective cytotoxicity against various cancer cell lines, including breast, pancreatic, prostate, and lung cancer cells (7,8,11,12). Studies have also found that these compounds can inhibit the growth and replication of cancer cells, trigger apoptosis (programmed cell death), and prevent angiogenesis (formation of blood vessels that supply tumors) (13−15).

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Scientific investigation have demonstrated the antimicrobial activity of *Annona muricata* against various bacteria, fungi, viruses, and parasites (16). Extracts from different parts of the plant have shown promising results in inhibiting the growth of pathogenic microorganisms and suppressing the activity of certain viruses (16−18). Research has also revealed the presence of antioxidative compounds in *Annona muricata*, which can help neutralize harmful free radicals in the body and protect against oxidative stress-related diseases (19,20). The seeds and leaves of the plant are reported to possess enzymatic antioxidants, including catalase and superoxide dismutase, and non-enzymatic antioxidants, including vitaminsC and E (19). Extracts from *Annona muricata* have demonstrated anti-inflammatory properties by reducing pro-inflammatory molecules, such as cytokines and enzymes involved in inflammation (21,22).

Additionally, some studies have suggested that *Annona muricata* may help regulate blood sugar levels by increasing insulin production or improving insulin sensitivity (6, 23). These effects can be attributed to various bioactive compounds found in the plant, such as flavonoids and alkaloids (6, 23, 24).

Furthermore, It is also of important to note that the plant *Annona muricata* has gained significant attention in scientific research due to its believed potential kidney and renal protective effects. In a recent study, Ojowu and his colleagues investigated the Ameliorative effect of leaf extract of *Annona muricata* on liver and kidney function in Carbon Tetrachloride (CCl4) induced rats, and the findings indicate that pretreatment with *Annona muricata* showed a protective effect in vivo in CCl4 compromised liver and kidneys (25). Similarly other studies results demonstrated that the leaves and fruit extract of *Annona muricata* significantly improved kidney function and reduced oxidative stress (26). These studies collectively suggest that *Annona muricata* leaves and fruit pulp possess beneficial properties for kidney and renal protection.

## MATERIALS AND METHODS

### Experimental rats

The rats used were adult albino male Wistar rats with weights between 100 g – 150 g. The rats were supplied by Mr. Silvanus Innih of the Anatomy Department, University of Benin, Benin City, housed in the Department of Biochemistry animal house, and were acclimatized for one week before the study. They were fed standard rat chow and water *ad libitum*. Written approval for the study was obtained from the Research Ethics Committee Guideline Principles on Handling of Animals of the College of Medicine, University of Benin (CMR/REC/2014/57), and was strictly adhered to (27).

### Preparation of *Annona muricata* crude (AMC) extracts

A large quantity of fresh parts of the plant was collected from trees in household gardens in Benin City and around the University of Benin vicinity, Edo state, Nigeria. The plant was identified by Dr. Bamidele of the Department of Plant Biology and Biotechnology, University of Benin, and authenticated by Professor Idu of the same Department. A voucher specimen number UBHa 0205 was deposited at the Herbarium of the Department of Plant Biology and Biotechnology, University of Benin. The samples (leaves, roots, stem bark, and pulp) were washed and pulverized separately after drying at room temperature (about 25 $^{o}$C) for 4 weeks. Each pulverized plant sample was macerated in methanol for 48 hours after which it was filtered through cheesecloth. The obtained extracts were then concentrated *in vacuo* using a rotary evaporator to obtain viscous gels which were air-dried to gel-like solids. The gel-like crude methanolic extracts obtained from the various parts of the plant were reconstituted to obtain a stock solution using distilled-deionized water as solvent. Each reconstituted crude extract was stored in small-capped plastic containers in a refrigerator at -4 $^{o}$C until used.

### Administration of extracts

The extracts were administered with the aid of a gavage, acting as an oro-gastric tube. Utmost care was taken not to inflict oral or oesophageal injuries on rats.

### Sub-chronic toxicity assessment

During the period of usage, extracts were administered to the rats based on calculated doses per weight of rat (*i.e*. equivalent volume). These dose calculations were done weekly per weight of rats for the sub-chronic studies, as the weekly weights of the rats per group were recorded *i.e*. day 0, day 7, day 14, day 21, and day 28. Untreated rats (group one) served as the control and were administered 2 ml of distilled water (28).

### Experimental Protocol for sub-chronic Toxicity Studies

Various methanolic extracts of the plant parts (fruit pulp, leaf, stem-bark, and root-bark) were administered at increasing doses from 100 mg/kg (group 2), 200 mg/kg (group 3), 400 mg/kg (group 4), 600 mg/kg (group 5) and 800 mg/kg (group 6). The group 1 rats were given 2 ml of distilled water (0 mg/kg) and served as the control. Each group had six (6) rats each.

### Biochemical assays of blood (plasma) parameters

Assays were done using ready-to-use standard assay kits from Randox® (Randox Laboratory, UK), including, aspartate aminotransferase (AST), alanine aminotransferase (ALT) (29), bilirubin (BIL) (30), total proteins (31), albumin (Bromocresol-Green as described by 32). Alkaline phosphatase activity was assayed using a ready-to-use kit from TECO (TECO Diagnostic, USA) (33). L- γ-Glutamyl transferase (GGT) was assayed by the method described by (34).

**Tissue protein and LDH determination**

Following the sacrifice of the rats, kidney tissues (0.4mg) were excised and collected into a sample bottle containing phosphate buffered (pH=7.4) normal saline, and subsequently homogenized, centrifuged and the supernatant collected into another plain sample bottle for onward assays. Tissue protein assay, lactate dehydrogenase activity (LDH), and glucose-6-phosphate dehydrogenase activity (G6PDH) activities were determined using standard assay kits from Randox® (Randox Laboratory, UK).

**Tissue processing, staining, and histology**

The liver tissues were harvested, weighed (to determine the hepatosomatic index, HIS), fixed in 10% formal saline, processed, and stained Tissue section photomicrographs were obtained and examined under the Leica DM750 research microscope with a digital camera (LeicaCC50) attached, with magnifications of x40 and x100.

$$Hepatosomatic\ index\ (HIS = \frac{Liver\ weight}{Body\ weight} \times 100$$

**Statistical analysis**

Data were entered into the Microsoft Excel spreadsheet (v.10) before descriptive analysis. The data are presented as mean ± SEM and were analyzed using Duncan's multiple range analyses of variance, ANOVA. Correlation analyses were done using Pearson's correlation ($p$=0.05) of the Statistical Package for Social Sciences, SPSS®, Version 21.0, IBM Corp., Armonk, NY, USA. Values of $p$< 0.05 were considered significant.

## RESULTS

**Hepatosomatic index (HIS) fold changes**

Figure 1 shows the Hepatosomatic index (HIS) fold changes of the various treatment. The result revealed that the group treated with the fruit pulp and the leaf extracts were most significantly influenced, disproportionately, especially at the lower doses of administration (100 mg/Kg); the group treated with the leaf extract increased significantly ($p \leq 0.05$) at 100 mg/kg with a progressive decrease with increasing dose, while the group treated with the fruit pulp decreased at the 100 mg/kg with a progressive increase with increasing dose.

**Effects of administration of fruit extract of *Annona muricata* on liver function parameters**

Table 1 shows the effect of administration of varying doses of fruit extracts of *Annona muricata* (AMC) on liver function parameters. The result showed that a significant increase($p \leq 0.05$) was observed for plasma ALP activity, Total bilirubin, and un conjugated bilirubin concentration for all the varying doses administered to the rats whereas a non-significant decrease ($p \geq 0.05$) was observed for the plasma AST and conjugated bilirubin levels. A significant reduction ($p \leq 0.05$) in ALT activities was noted for the groups treated with 100, 400, 600, 800mg/kg body weight when compared with the control

The results of table 2 shows that there was a significant increase($p \leq 0.05$) in plasma γ-glutamyl transferase, Total protein and globulin levels while non-significant differences ($p \geq 0.05$) was observed for albumin, and a significant decrease ($p \leq 0.05$) in liver tissue protein, and LDH levels when compared with the control group.

**Effects of administration of leaf extract of *Annona muricata* on liver function parameters**

Table 3 shows the result of administration of varying doses of leaf extract of *Annona muricata* on liver function parameters. A significant decrease ($p \leq 0.05$) was observed for AST, ALT (at the lower doses), and tissue protein (for 200, 400 and 800mg/kg body weight). A significant increase ($p \leq 0.05$) was also observed for ALT activity at the higher doses (600 and 800mg/kg body weight administered groups). Wheras, a non-significant change ($p \geq 0.05$) was observed for the concentration of total bilirubin, conjugated bilirubin and unconjugated bilirubin.

The result of table 4 shows that a significant decrease ($p \leq 0.05$) was also observed for liver tissue protein and lactate dehydrogenase level γ-glutamyl transferase (for 100, 400 and 800mg/kg body weight) while a non-significant differences ($p \geq 0.05$) was observed for the Total protein and globulin levels.

**Effects of administration of stem bark of *Annona muricata* on liver function parameters**

Table 5 and 6 shows the effect of administration of stem bark of *Annona muricata* on liver function parameters. A non-significant differences ($p \geq 0.05$) was observed for all the liver parameters (ALT, AST, ALP, Total, conjugated and unconjugated bilubin, plasma γ-glutamyl transferase, Total protein, albumim, liver tissue protein and lactate dehydrogenase levels).

**Effects of administration of root bark of *Annona muricata* on liver function parameters**

Table 7 and 8 shows the effect of administration of root bark of *Annona muricata* on liver function parameters. A non-significant differences ($p \geq 0.05$) was observed for the markers ALT, AST, ALP activities, plasma γ-glutamyl transferase, Total protein, albumim, liver tissue protein and lactate dehydrogenase levels while slight alterations were observed for the total bilirubin, conjugated and unconjugated levels when compared with the control groups.

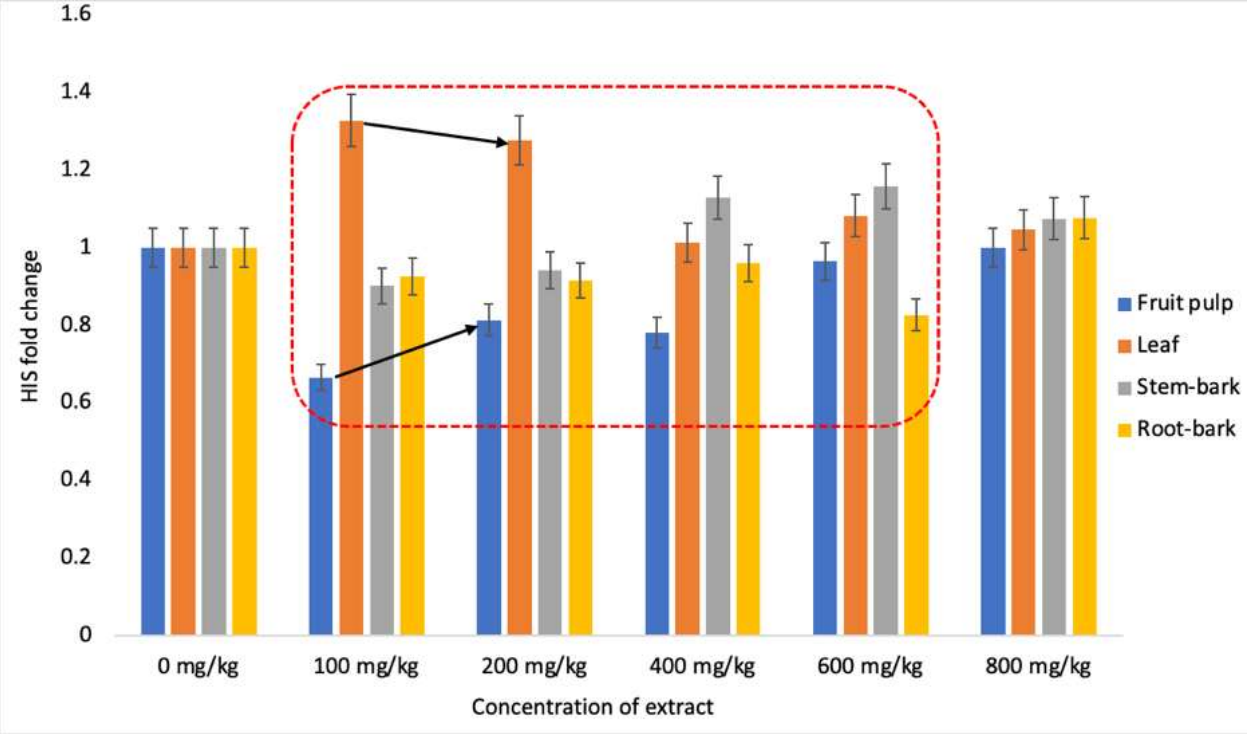



Figure 1. Hepatosomatic index fold changes due to *Annona muricata* extract treatments

**Table 1. Liver function parameters of rats administered fruit extract of *Annona muricata***

| GROUPS | Total bilirubin (mg/dl) | Conjugated bilirubin (mg/dl) | Unconjugated bilirubin (mg/dl) | AST (U/I) | ALT (U/I) | ALP (U/L) |
|---|---|---|---|---|---|---|
| Group 1(0mg/kg) | 0.04±0.04$^a$ | 0.03±0.06$^a$ | 0.01±0.04$^a$ | 45.10±1.20$^a$ | 27.07±4.00$^a$ | 10.00±5.00$^a$ |
| Group 2 (100mg/kg) | 0.09±0.01$^b$ | 0.04±0.08$^a$ | 0.05±0.10$^b$ | 42.02±0.61 $^b$ | 12.05±2.00$^b$ | 12.33±8.00$^a$ |
| Group 3 (200mg/kg) | 0.08±0.05$^b$ | 0.02±0.01$^a$ | 0.06±0.31$^b$ | 51.06±2.00$^b$ | 23.02±2.20$^b$ | 16.83±40.80$^b$ |
| Group 4 (400mg/kg) | 0.07±0.05$^b$ | 0.04±0.01$^a$ | 0.03.18±.10 | 41.01±0.20$^b$ | 16.05±1.00$^b$ | 28.33±35.00$^b$ |
| Group 5 (600mg/kg) | 0.10±0.09$^b$ | 0.03±0.006$^a$ | 0.07±2.00$^b$ | 32.03±1.00$^b$ | 18.97±1.00$^b$ | 17.00±77.00$^b$ |
| Group 6 800mg/kg | 0.06±0.06 | 0.01±0.06 | 0.05±3.00$^b$ | 21.07±3.00$^b$ | 16.52±3.00$^b$ | 32.83±41.00$^b$ |

Values are represented as mean±SEM (n=6). Means with different superscripts are significantly different ($p<0.05$) down the column by one way Duncan's multiple ranges ANOVA, with the control (group A) taking the superscript 'b' alphabet

**Table 2. Liver function parameters of rats administered fruit extract of *Annona muricata***

| GROUPS | GGT (U/L) | Plasma total protein (g/l) | ALB (g/dl) | GLB (g/l) | Liver tissue protein (mg/g) | Liver LDH (u/L) |
|---|---|---|---|---|---|---|
| Group 1 (0mg/kg) | 0.45±0.50$^a$ | 13.77±0.30$^a$ | 4.90±1.0$^a$ | 8.87±2.00$^a$ | 1.50±1.00$^a$ | 0.95±0.40$^a$ |
| Group 2 (100mg/kg) | 0.78±0.10$^b$ | 14.37±0.20 | 5.00±0.7$^a$ | 9.37±2.00$^a$ | 1.70±0.70$^b$ | 0.60±0.30$^b$ |
| Group 3 (200mg/kg) | 0.29±0.30 | 37.33±2.00$^b$ | 3.70±2.0$^b$ | 33.63±3.00$^b$ | 1.23±2.00$^b$ | 0.88±0.70$^a$ |
| Group 4 (400mg/kg) | 0.94±0.10$^b$ | 39.78±0.50$^b$ | 4.80±1.0$^a$ | 34.98±1.00$^b$ | 1.08±1.00$^b$ | 0.28±0.50$^b$ |
| Group 5 (600mg/kg) | 0.22±0.40$^b$ | 38.00±3.00$^b$ | 4.00±3.0$^a$ | 34.00±1.00$^b$ | 1.00±3.00$^b$ | 0.82±0.50$^a$ |
| Group 6 (800mg/kg) | 0.62±0.40$^b$ | 41.78±1.00$^b$ | 4.10±1.0$^a$ | 37.68±1.63$^b$ | 1.70±1.00$^b$ | 0.77±30.00$^b$ |

Values are represented as mean±SEM (n=6). Means with different superscripts are significantly different ($p<0.05$) down the column by one way Duncan's multiple range ANOVA, with the control (group A) taking the superscript 'b' alphabet.

**Table 3. Liver function parameters of rats administered leaf extract of *Annona muricata***

| GROUPS | Total bilirubin (mg/dl) | Conjugated bilirubin (mg/dl) | Unconjugated bilirubin (mg/dl) | AST (U/I) | ALT (U/I) | ALP (IU/L) |
|---|---|---|---|---|---|---|
| Group 1 (0mg/kg) | 0.04±3.00[a] | 0.02±0.10[a] | 0.02±3.00[a] | 12.78±1.00[a] | 8.12±3.00[a] | 8.55±4.00[a] |
| Group 2 (100mg/kg) | 0.02±1.00[a] | 0.01±0.10[a] | 0.01±3.00[a] | 9.78±1.00[b] | 5.85±2.00[b] | 9.67±1.00[a] |
| Group 3 (200mg/kg) | 0.05±1.00[a] | 0.03±1.00[a] | 0.02±5.00[a] | 4.40±1.00[b] | 5.91±2.00[b] | 18.83±5.00[b] |
| Group 4 (400mg/kg) | 0.01±0.10[a] | 0.03±1.00[a] | 0.07±2.00[b] | 8.83±1.00[b] | 3.53±3.00[b] | 9.83±3.00[a] |
| Group 5 (600mg/kg) | 0.04±0.30[a] | 0.02±0.10[a] | 0.02±2.00[a] | 9.60±1.00[b] | 13.53±3.00[b] | 13.00±4.00[b] |
| Group 6 (800mg/kg) | 0.05±0.10[a] | 0.03±1.00[a] | 0.02±5.00[a] | 5.03±2.00[b] | 13.75±3.00[b] | 22.17±63.00[b] |

Values are represented as mean±SEM (n=6). Means with different superscripts are significantly different ($p<0.05$) down the column by one way Duncan's multiple range ANOVA, with the control (group A) taking the superscript 'b' alphabet.

**Table 4. Liver function parameters of rats administered leaf extract of *Annona muricata.***

| GROUPS | GGT (U/L) | Plasma total protein (g/l) | ALB (g/dl) | GLB (g/l) | Liver tissue protein | Liver LDH |
|---|---|---|---|---|---|---|
| Group 1 (0mg/kg) | 5.27±0.10[a] | 12.57±0.10[a] | 6.5±1.00[a] | 6.07±1.00[a] | 7.83±0.30[a] | 9.03±1.00 [a] |
| Group 2 (100mg/kg) | 4.81±0.10[b] | 12.92±1.00[a] | 3.5±0.70 [b] | 9.42±3.00[b] | 8.85±3.00 | 8.37±1.00 |
| Group 3 (200mg/kg) | 4.87±0.90[a] | 10.72±2.00[b] | 4.7±2.00[a] | 6.02±2.00 | 3.67±6.21[b] | 4.15±0.40[b] |
| Group 4 (400mg/kg) | 4.88±0.20[a] | 13.55±0.30[a] | 4.8±1.00[a] | 8.75±1.00[b] | 4.83±0.70[b] | 2.72±0.30[b] |
| Group 5 (600mg/kg) | 5.07±0.20[a] | 13.57±0.30[a] | 4.1±3.00 [a] | 9.47±2.00[b] | 4.00±1.00[b] | 9.25±1.00[a] |
| Group 6 (800mg/kg) | 5.72±0.40[a] | 12.54±1.00[a] | 4.4±1.00[a] | 8.14±1.63[b] | 8.95±3.00[b] | 9.18±0.40[a] |

Values are represented as mean±SEM (n=6). Means with different superscripts are significantly different ($p<0.05$) down the column by one way Duncan's multiple range ANOVA, with the control (group A) taking the superscript 'b' alphabet.

**Table 5. Liver function parameters of rats administered stem-bark extract of *Annona muricata.***

| GROUPS | Total bilirubin (mg/dl) | Conjugated bilirubin (mg/dl) | Unconjugated bilirubin (mg/dl) | AST (U/I) | ALT (U/I) | ALP (IU/L) |
|---|---|---|---|---|---|---|
| Group 1 (0mg/kg) | 0.04±0.10[a] | 0.02±0.00[a] | 0.02±0.10[a] | 11.28±0.00[a] | 10.07±0.00[a] | 18.15±0.00[a] |
| Group 2 (100mg/kg) | 0.04±0.00[a] | 0.02±0.10[a] | 0.02±0.00[a] | 10.22±0.60[a] | 11.65±4.00[a] | 19.30±1.00[a] |
| Group 3 (200mg/kg) | 0.03±1.00[a] | 0.01±1.00[a] | 0.02±0.30[a] | 14.00±1.00 | 9.96±0.10[a] | 18.90±0.01[a] |
| Group 4 (400mg/kg) | 0.03±0.00[a] | 0.02±0.10[a] | 0.01±0.60[a] | 10.65±0.07[a] | 11.73±0.00[a] | 19.10±0.00[a] |
| Group 5 (600mg/kg) | 0.04±0.30[a] | 0.03±0.10[a] | 0.02±0.01[a] | 10.80±1.00[a] | 12.10±0.90[a] | 17.00±0.20[a] |
| Group 6 (800mg/kg) | 0.05±0.10 | 0.03±0.20[a] | 0.02±1.00[a] | 11.00±0.30[a] | 12.89±1.00[a] | 19.39±0.80[a] |

Values are represented as mean±SEM (n=6). Means with different superscripts are significantly different ($p<0.05$) down the column by one way Duncan's multiple ranges ANOVA, with the control (group A) taking the superscript 'b' alphabet.

**Table 6. Liver function parameters of rats administered stem-bark extract of *Annona muricata***

| GROUPS | GGT (U/L) | Plasma total protein (g/l) | ALB (g/dl) | GLB (g/l) | Liver tissue protein | Liver LDH |
|---|---|---|---|---|---|---|
| Group 1 (0mg/kg) | 6.21±0.30[a] | 14.22±0.10[a] | 7.66±2.00[a] | 6.56±0.00[a] | 8.61±0.30[a] | 10.32±0.02[a] |
| Group 2 (100mg/kg) | 7.33±0.00[a] | 10.39±0.00[b] | 6.37±3.00[b] | 4.02±0.20[a] | 8.39±3.00[a] | 9.47±0.00[a] |
| Group 3 (200mg/kg) | 6.66±0.20[a] | 11.43±0.00[b] | 7.98±0.00[a] | 3.45±0.10[b] | 8.18±6.21[a] | 10.00±0.09[a] |
| Group 4 (400mg/kg) | 6.09±0.00[a] | 12.89±0.20[a] | 6.53±1.00[a] | 6.36±0.08[a] | 7.98±0.70[a] | 9.83±0.10[a] |
| Group 5 (600mg/kg) | 5.37±1.00[a] | 12.66±0.00[a] | 6.93±0.00[a] | 5.73±0.00[a] | 7.56±1.00[a] | 9.12±0.00[a] |
| Group 6 (800mg/kg) | 6.81±0.00[a] | 14.81±0.00[a] | 7.26±0.50[a] | 7.55±0.00[a] | 8.31±3.00[a] | 8.29±2.00[b] |

Values are represented as mean±SEM (n=6). Means with different superscripts are significantly different ($p<0.05$) down the column by one way Duncan's multiple range ANOVA, with the control (group A) taking the superscript 'b' alphabet.

**Table 7. Liver function parameters of rats administered root-bark extract of *Annona muricata***

| GROUPS | Total bilirubin (mg/dl) | Conjugated bilirubin (mg/dl) | Unconjugated bilirubin (mg/dl) | AST (U/I) | ALT (U/I) | ALP (IU/L) |
|---|---|---|---|---|---|---|
| Group 1 (0mg/kg) | 0.06±0.10$^a$ | 0.03±0.00$^a$ | 0.04±0.00$^a$ | 6.32±0.00$^a$ | 6.77±0.01$^a$ | 9.61±0.00$^a$ |
| Group 2 (100mg/kg) | 0.07±0.00$^a$ | 0.04±0.00$^a$ | 0.03±0.00$^a$ | 7.49±0.00 | 8.31±0.60$^b$ | 9.20±0.02$^a$ |
| Group 3 (200mg/kg) | 0.05±0.00$^a$ | 0.03±0.00$^a$ | 0.02±0.00$^a$ | 7.20±1.00$^a$ | 7.23±0.00$^a$ | 10.01±0.02$^a$ |
| Group 4 (400mg/kg) | 0.03±0.00$^b$ | 0.02±0.00$^a$ | 0.01±0.00$^b$ | 6.98±0.00$^a$ | 7.04±0.00$^a$ | 10.23±0.10$^a$ |
| Group 5 (600mg/kg) | 0.02±0.00$^b$ | 0.01±0.10$^a$ | 0.01±0.00$^b$ | 7.10±0.00$^a$ | 6.32±0.07$^a$ | 9.67±0.20$^a$ |
| Group 6 (800mg/kg) | 0.02±0.00$^b$ | 0.01±0.00$^a$ | 0.01±0.00$^b$ | 6.21±0.00$^a$ | 6.71±0.00$^a$ | 9.83±0.00$^a$ |

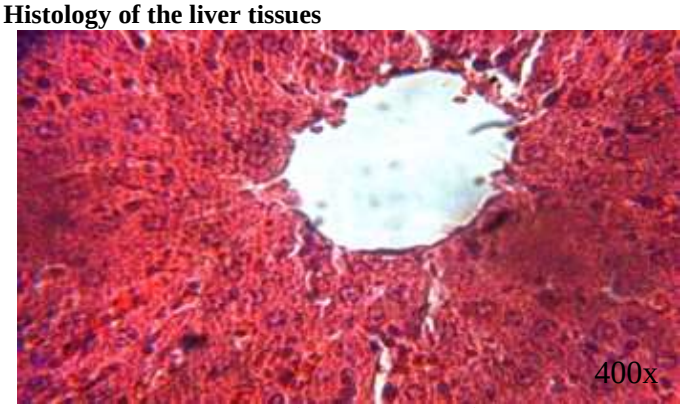
Values are represented as mean±SEM (n=6). Means with different superscripts are significantly different ($p<0.05$) down the column by one way Duncan's multiple range ANOVA, with the control (group A) taking the superscript 'b' alphabet.

**Table 8. Liver function parameters of rats administered root-bark extract of *Annona muricata***

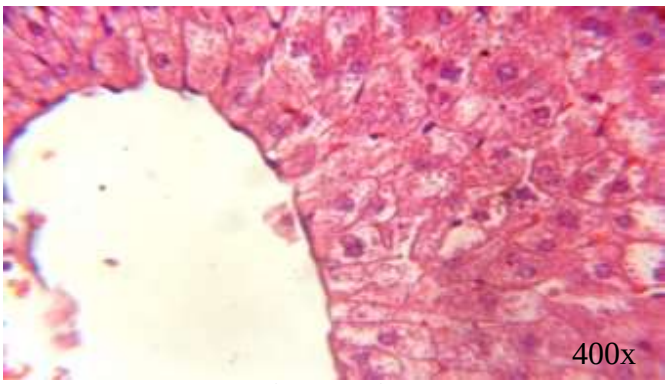
| GROUPS | GGT (U/L) | Plasma total protein (g/l) | ALB (g/dl) | GLB (g/l) | Liver tissue protein | Liver LDH |
|---|---|---|---|---|---|---|
| Group 1 (0mg/kg) | 3.24±0.00$^a$ | 10.42±0.02$^a$ | 7.10±0.30$^a$ | 3.32±2.00$^a$ | 5.98±0.00$^a$ | 6.72±1.00$^a$ |
| Group 2 (100mg/kg) | 3.65±0.10$^a$ | 10.98±0.00$^a$ | 6.23±0.00$^a$ | 4.75±0.08$^a$ | 5.82±0.00$^a$ | 7.81±1.00$^b$ |
| Group 3 (200mg/kg) | 3.19±0.00$^a$ | 10.46±0.00$^a$ | 5.36±0.00$^a$ | 5.10±0.40$^b$ | 6.34±0.01$^a$ | 6.72±0.40$^a$ |
| Group 4 (400mg/kg) | 4.00±0.00$^a$ | 11.56±0.06$^a$ | 6.22±0.00$^a$ | 5.34±0.00$^b$ | 6.89±0.00$^a$ | 6.24±0.30$^c$ |
| Group 5 (600mg/kg) | 3.10±0.02$^a$ | 10.48±0.00$^a$ | 5.98±0.01$^a$ | 4.50±0.20$^a$ | 7.01±0.02$^c$ | 6.50±1.00$^a$ |
| Group 6 (800mg/kg) | 4.31±0.00$^b$ | 11.92±0.00$^a$ | 5.86±0.00$^a$ | 6.08±0.06$^b$ | 6.83±0.00$^a$ | 7.12±0.40$^a$ |

Values are represented as mean±SEM (n=6). Means with different superscripts are significantly different ($p<0.05$) down the column by one way Duncan's multiple range ANOVA, with the control (group A) taking the superscript 'b' alphabet.

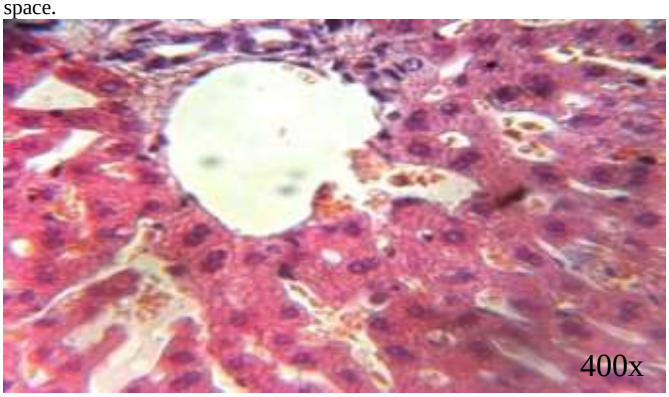
**Histology of the liver tissues**



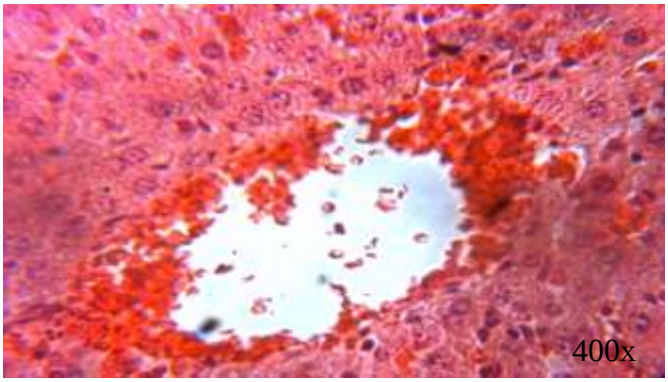

Plates 1. Photomicrographs of the liver tissue for group 1 (control) administered 2ml distilled water (only) at 400x magnifications showing a normal hepatocyte with visible centrioles with well-fenestrated sinusoidal space.



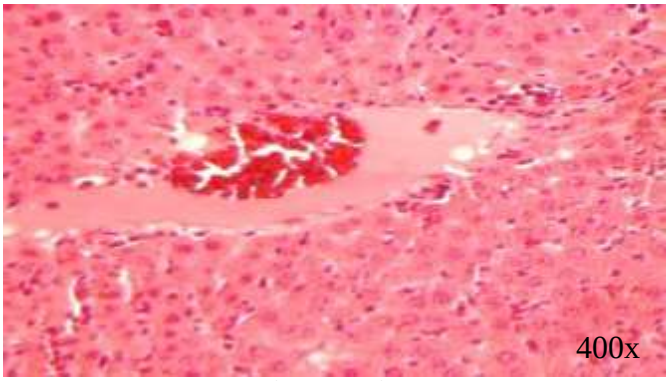

Plates 2. Photomicrographs of the liver for group 2 were administered 100mg/kg of fruit extract showing normal hepatocytes with visible centrioles without any marked pathological changes.



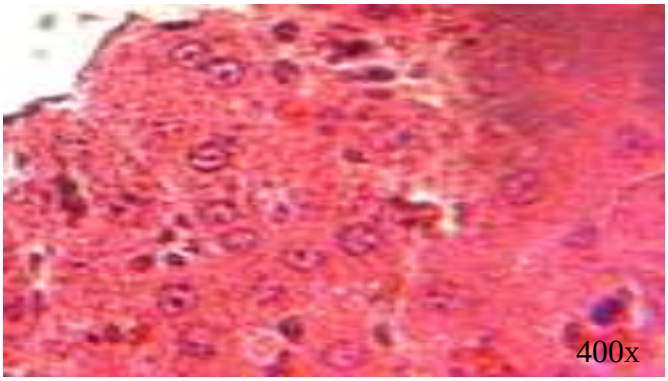

Plates 3. Photomicrographs of the liver for group 3 were administered 200mg/kg of fruit extract showing prominent hepatocytes with visible nuclei and centrioles with the no marked pathological changes.



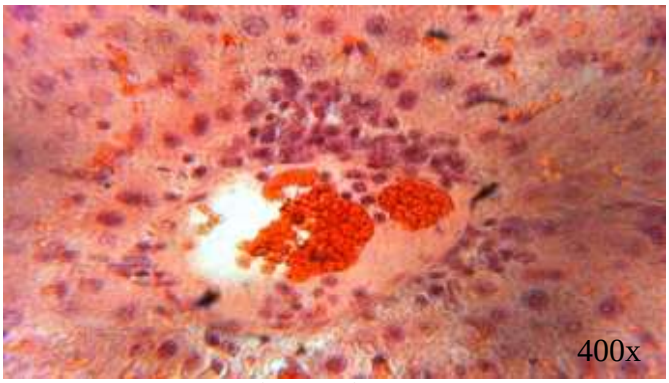

Plates 4. Photomicrographs of the liver for group 4 were administered 400mg/kg of fruit extract mild congestion in the centrioles and mild inflammatory processes characterized by mononuclear cell exudation (lymphocytes and histiocytes) in the portal and lobular compartments.



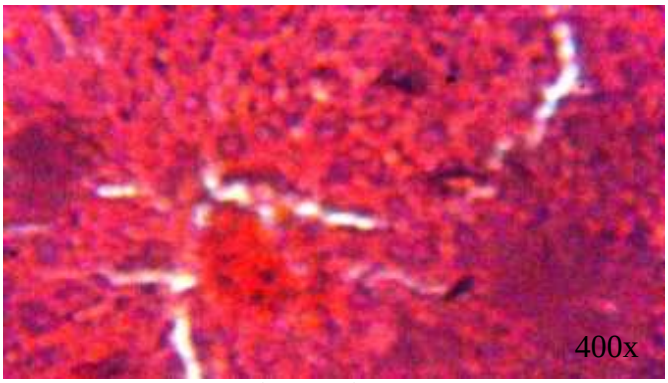

Plates 5. Photomicrographs of the liver for group 5 were administered 600mg/kg of fruit extract showing mild congestion in the centrioles and mild infiltrates of cells in the portal and lobular compartments.



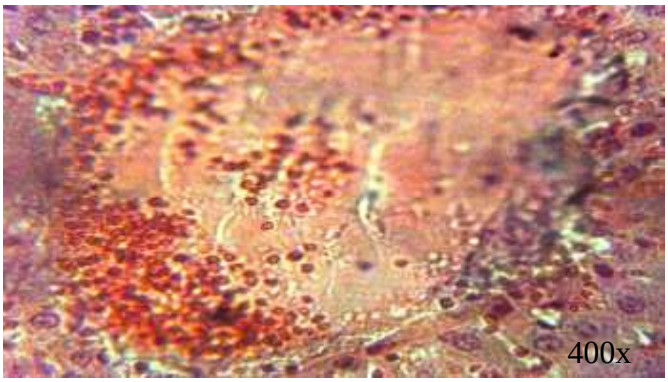

Plates 6. Photomicrographs of the liver for group 6 were administered with 800mg/kg of fruit extract showing erythrocytes and mononuclear cell infiltrates in the sinusoids.




Plates 7. Photomicrographs of the liver for group 6 were administered with 800mg/kg of leaf extract showing mononuclear cell infiltration in the vicinity of sinusoids and stromal fibrosis in the liver.




Plates 8. Photomicrographs of the liver for group 6 were administered with 800mg/kg of stem-bark extract showing exudates of inflammatory cells.




Plates 9. Photomicrographs of the liver for group 6 were administered with 800mg/kg of root-bark extract showing exudates of inflammatory cells

## DISCUSSION

Overall, this research aims to elucidate the influence of *Annona muricata* on liver function parameters, contributing to a better understanding of the plants' different morphological parts' potential hepatoprotective properties. The results obtained from this study may serve as a basis for further research to explore the mechanisms underlying the observed effects and potentially develop novel therapeutic interventions for liver-related disorders.

In clinical practice, liver function tests are frequently used to check for liver disease, track the development of an existing condition, and assess the effects of potentially hepatotoxic medications (35). High and low concentrations of conjugated bilirubin may indicate various conditions related to liver function and bile metabolism. Elevated levels of conjugated bilirubin (hyperbilirubinemia) can suggest liver diseases such as hepatitis, cirrhosis, or obstructive jaundice (36). From the result of the study, it was observed that the fruit, leaf, and stem bark of *Annona muricata* extracts showed no significant differences when compared to the normal control. However, the groups (400 mg/kg, 600 mg/kg, 800 mg/kg) given *Annona muricata* root bark extracts showed a slightly significant reduction in the levels of conjugated bilirubin when compared to the normal control group. Our findings on the fruit extracts were not consistent with the report of Usunobun *et al*. (37) in their study on the hepatoprotective effect of *Annona muricata* on acetaminophen-induced liver toxicity. They reported that treatment with extracts from the leaves resulted in a significant reduction in the high bilirubin caused by acetaminophen induction.

Comparing the effects of the extract of the various morphological parts of the plant *Annona muricata* on the concentration of unconjugated bilirubin. It was observed that only the groups administered *Annona muricata* root extract had a significant effect on the concentration of unconjugated bilirubin. The root extracts significantly reduced the concentration of unconjugated bilirubin. Hence, the result indicates that *Annona muricata* root bark extracts may be

therapeutic in reducing the concentration of unconjugated bilirubin in liver disorders. Elevated levels of unconjugated bilirubin, known as hyperbilirubinemia, can indicate various liver disorders, such as Gilbert's syndrome, hemolysis (excessive breakdown of red blood cells), or certain types of hepatitis.Interestingly there was no significant change in all the groups given the different morphological parts of *Annona muricata* extracts except the groups administered 600 mg/kg and 800 mg/kg of *Annona muricata* root bark extracts.

AST is primarily used as a marker for liver health. Elevated levels of AST in the blood can indicate liver damage or injury, as AST is normally found in the liver. High levels of AST might suggest conditions like hepatitis, cirrhosis, or drug-induced liver injury. Analyzing the effects of the different parts of *Annona muricata* extracts on the concentration of aspartate aminotransferase (AST). All the groups administered the *Annona muricata* leaf extracts showed non-significant changes in the blood levels of AST except for the 800 mg/kg body weight groups. Similarly, the groups administered 100 mg/kg, 400 mg/kg, 600 mg/kg, and 800 mg/kg of *Annona muricata* fruit extract showed no significant reduction in the concentration of AST. The results observed from the groups administered *Annona muricata* stem-bark extract varied differently, the 100 mg/kg group significantly reduced the concentration of AST, while there was no significant change in the other groups.

Alkaline phosphatase (ALP) is an enzyme found in various tissues throughout the body. Elevated blood ALP levels can indicate issues such as liver or bone disorders, gallstones, or certain cancers. *Annona muricata* fruit and leaf extracts showed alterations in the concentration of ALP differing on each dose when compared to their normal control groups (Tables 1 and 3). On the other hand, the groups administered stem and root bark *Annona muricata* extracts showed non-significant changes in the concentration of ALP (Tables 5 and 7).

Alanine aminotransferase (ALT) is an enzyme found in the liver. It's a valuable marker for diagnosing liver-related issues. Elevated ALT levels in the blood are used to indicate different liver diseases and disorders. Generally, the groups administered *Annona muricata* fruit extracts (100 mg/kg, 400 mg/kg, 600 mg/kg, 800 mg/kg) significantly reduced the concentration of blood ALT, among other groups that also showed the same effect including 800 mg/kg group of the leaf extract. Non-significant reduction was observed for all other extracts administered groups. The findings in this study agree with those of Usunobun *et al.*, (38) who reported on the modulatory effect of ethanolic leaf extract of *Annona muricata* pre-treatment on liver damage induced by Dimethylnitrosamine (DMN) in Rats.

From the study, we observed a significant reduction in the concentration of LDH (Lactate dehydrogenase) in groups administered *Annona muricata* fruits and leaf extract,

Albumin (ALB) is a protein produced by the liver and plays a crucial role in maintaining various bodily functions. Reduction in serum albumin levels is suggestive of liver diseases (39). Except for the groups treated with 200 mg/kg of leaf and fruit, which gave a significant reduction, all other groups administered *Annona muricata* extracts showed non-significantly reduction in the concentration of ALB (Tables 2, 4).

Liver tissue proteins, like enzymes and markers, are often measured in blood tests to assess liver function. A significant reduction in the concentration of liver tissue proteins was observed in groups administered *Annona muricata* leaf extracts. A significant alterations were observed in all groups given the *Annona muricata* fruit extracts (Tables 2, 4, 6 and 8)

GLB (Globulin) in the blood can be used as a marker to indicate liver damage or disease, often associated with conditions like hepatitis, cirrhosis, or alcohol abuse. A significant increase was observed in groups 3 - 6 administered *Annona muricata* fruit extracts, also for the group treated with 200, 400mg/kg body weight of root bark extract. Non-significant changes were observed for all other groups (Tables 2, 4, 6, 8).

Gamma-glutamyl transferase (GGT) is an enzyme found in various tissues, especially the liver and kidneys. A significant decrease in the concentration of GGT was in groups given Annona *muricata* leaf (100 mg/kg, 200 mg/kg, and 400 mg/kg) and root-bark (200 mg/kg *Annona muricata.* Conversely, we noticed a significant increase in GGT concentration in groups given 100, 400, and 800mg/kg body weight of fruit extracts. This finding is consistent with a report by Usunobun *et al.* (38).

Comparing the effects of the different morphological parts of *Annona muricata* plant on the concentration of plasma total protein. In this study, we recorded a significant increase in total protein concentration in group 2 – 6 administered *Annona muricata* fruit extracts when compared with the normal control (table 2). Similarly significant increase was also recorded for groups 4 and 5 administered *Annona muricata* leaf extracts and groups given 100 mg/kg, 400 mg/kg, 600 mg/kg, and 800mg/kg of *Annona muricata* root bark extracts. On the contrary, the groups that were given stem bark all showed a significant reduction in plasma protein concentration, the same as group 3 (200 mg/kg) which was given *Annona muricata* leaf extracts.

Several studies have established the hepatoprotective effects of extracts of *Annona muricata* and these advantageous effects have been linked to the rich content of phytochemicals such as tannins, alkaloids, saponins, and ascorbic acids (40, 41).

Histological examination of the liver tissues showed no pathological changes in the lower doses (100 and 200 mg/kg body weight) treated groups while mild inflammatory processes characterized by mononuclear cell exudation

(lymphocytes and histiocytes) in the portal and lobular compartments were evident in the high doses (400, 600 and 800 mg/kg body weight) treated groups(plates 1−9). The presence of inflammatory cells in the liver is important in maintaining tissue and organ homeostasis.

**Conclusion:** This study has established that mild alterations in the liver markers (ALT, AST, ALP, GGT, TP, Bilirubin, LDH) could result from administration of varying doses of the different morphological parts of *Annona muricata* (leaf, fruits, root, stem bark). However, we observed that these alterations in the liver markers only produced mild changes on histological examination of the liver tissues which may not be detrimental in any way.

**Conflict of interest:** Authors declare no conflict of interests

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