Hematopoietic and enzyme modulatory effects of aqueous stem bark extract of *Annona Muricata*

Okoye J.O.,1* Effiong G.S.2

1Histopathology Specialty, Medical Laboratory Science Department, School of Public and Allied Health, Babcock University, Ilishan-Remo, Ogun State, Nigeria.
2Medical Laboratory Science Department, Faculty of Health Sciences, Madonna University, Elele Campus, Rivers State, Nigeria.

*corresponding author: okoyej@babcock.edu.ng

**Abstract:** This study evaluated the effects of aqueous stem bark extract of *Annona muricata* on the liver of albino rats. A total of 24 male albino rats, randomly divided into 4 groups: 1(control), 2, 3 and 4, were orally administered 0, 25, 50 and 100 mg/kg body weights of the extract, respectively for 14 days. Blood samples were analyzed using the Reitman and Frankel, Cyanmethaemoglobin methods. Comparing the control and test groups, results showed significantly increased Hb (16.40 ± 2.48 to 22.74 ± 3.83), PCV (49.20 ± 7.40 to 68.20 ± 11.52), organ weight (7.65 ± 1.14 to 8.17 ± 0.93) and relative weight (5.12 to 5.64). Higher levels of ALP (492.50 ± 169.27 to 568.00 ± 265.67) were observed in group 3 and 4 while lower level was observed in group 2 (397.16 ± 188.80). Lower levels of ALT (22.50 ± 11.18 to 17.67 ± 3.56) and LDH (145.00 ± 10.41 to 88.83 ± 57.81) were also observed when the control group was compared with the test groups (p>0.05). Liver microscopy revealed mild to moderate necrosis in group 3 and 4. The study suggests that *Annona muricata* was non-toxic at 25 mg/kg and may possess hematopoietic and hepatoprotective effects.

### 1.0 INTRODUCTION

*Annona muricata* is a species of the genus *Annona* of the custard apple tree family, Annonaceae, known mostly for its edible fruit. The fruit is usually called soursop due to its slightly acidic or sour taste when ripe. *A. muricata* is native to the Caribbean and Central America but is now widely cultivated and in some areas, becoming invasive in tropical climates throughout the world it is commonly known as soursop (Morton and Julia, 2007). The soursop also called graviola from the Spanish historian Gonzalo Fernández de Oviedo y Valdés in 1526 and was spread around the world by the Spanish explorers (Lannuzel et al., 2003). The raw nutritional value of soursop per 100g includes Vit B1 (thiamine), B2 (riboflavin), B3, and C, Pathothenic acid, Niacin, Folate, Choline, protein and trace metals such as calcium, Iron, Magnesium, Phosphorus, Potassium, sodium and zinc (Champy et al., 2005).

The bark, leaves, and roots are considered sedative, antispasmodic, hypotensive anthelmintic, antiparasitic, antipyretic, sedative, antispasmodic, nervine, hypotensive, anticonvulsant, cardio depressant and digestive (Antoun, 1993; Feras, 1999; Lannuzel et al. 2003). The The bark used for Asthenia, asthma, childbirth, cough, heart tonic, hypertension, nervine, parasites, sedative etc (Feras, 1999). The stem and bark in an ethanol extract at 1 mg/ml had in vitro activity against herpes simplex 1, while the root had activity against herpes simplex type 2 (Feng, 1962). Many of the acetogenins have demonstrated selective toxicity to tumor cells (at very low dosages): lung carcinoma cell lines; human breast solid tumor lines; prostate adenocarcinoma; pancreatic carcinoma cell lines; colon adenocarcinoma cell lines; liver cancer cell lines; human lymphoma cell lines; and multi-drug resistant human breast adenocarcinoma (Lannuzel et al., 2003). Acetogenins act by inhibiting NADH oxidase in the plasma membranes of cancer cells. This enzyme is only transiently expressed in ‘normal healthy’ cells. By inhibiting this enzyme cellular ATP is depleted which in turn causes inhibition of oxidative phosphorylation and cancer cell growth. The acetogeninnanonacin is able to induce apoptotic cell death. It enhanced the expression of Bax and Bad (Feng, 1962).

The seeds and bark are considered toxic and contain a number of compounds and potentially poisonous alkaloids such as anonaine, muricine, and hydrocyanic acid. This can cause inflammation problems and neurotoxic effects (Tattersfield and Padma, 1940; Lannuzel et al., 2003). Researchers have suggested that these alkaloids might be linked to atypical Parkinson’s disease in countries where the seeds are employed as a common herbal parasite remedy (Hasrat et al., 1997). The aim of this study was to determine the dosage at which...
aqueous stem bark extract of *Annona muricata* is detrimental to the body system in relation to some haematological and biochemical parameters, and microanatomical architecture of the liver in albino rats.

2.0 MATERIALS AND METHODS

**Study area**

The study was carried out at the animal farm in the department of Medical Laboratory Science, Faculty of Health Sciences, Madonna University Elele, Rivers state, Nigeria. The study area is located in the tropics (with the mean daily temperature of 29 °C) at the southern part of Nigeria; latitude 5 27-5 31N and longitude 6 55-7 85E (Gobo, 1988). It is bordered by four neighboring communities namely Isiokpo, Umuagwo, Ahoda, and Onoku.

**Ethical approval**

The research received ethical approval from the ethics committee of the department of Medical Laboratory Science, Faculty of Health Sciences, Madonna University, Nigeria (FHS/MLS/10/041). The experiment was conducted in accordance with the Guidelines of the U.S. National Institute of Health (NIH, 1985) on the care and use of laboratory animals and also in accordance with the principles of Good Laboratory Procedure (WHO, 1998).

**Test sample**

Fresh large quantity of stem bark of *Annona muricata* tree was gotten from Amucha village in Orlu local government of Imo state Nigeria. The botanic identification and authentication was carried out in faculty of pharmacy Madonna University, Elele campus by a taxonomist and a sample keep at the herbarium (MU/PHGSY/05/001).

**Preparation of extract**

Fresh stem bark of *Annonaceae muricata* was cut from the tree, dried for one week until there were no evidence of obvious moisture in them, the dried stem bark was grinded into powered form. Six hundred (600) ml of distilled water was added to 100 grams of the ground stem bark and allowed to stand in a refrigerator for 72hrs. The supernatant was decanted into a beaker, filtered using filter papers and the water evaporated to dryness in an oven. After drying, the powder form of the extract was raped in aluminum foil and preserved in a refrigerator (Francis, 2000; Cheng et al., 2014).

**Phytochemical analysis**

The test carried out was based on procedures outlined by Harbourne (1973) and Evans (2002). Estimation of carbohydrates was carried out by the Molisch’s test, Saponins by Frothing test, Tannins by Ferric chloride test, Flavanoids by Ammonium test, Glycosides by Fehling’s solution test, Protein by Millon’s test, Steroids by the chloroform-sulphuric acid test and alkaloids by Mayer-wagner’s test.

**Animals handling**

The experimental animals used for this study were obtained from the animal house of Madonna University, Elele, Nigeria housed in wire meshed cage under standard conditions (temperature 25 - 29°C, 12 hours light and 12 hours darkness cycles).

**Experimental design**

This experimental controlled study was carried out in Madonna University, Elele Campus, River State; A total of 24 Male albino rats weighing between 110-202g were used as animal model, housed in four meshed cages containing 6 rats in each. The animals were allowed to acclimatize for two weeks before the experiment began. They were allowed to feed on standard rat chow and water freely throughout the period the experiment. Administration of the aqueous extract of *Annona muricata* stem bark was given orally; Group 1 received 2ml of distilled water, Group 2: received 25 mg/kg body weight of *Annona muricata* extract, Group 3: received 50 mg/kg body weight of *Annona muricata* extract while Group 4: received 100 mg/kg body weight of *Annona muricata*. The experiment lasted for 14 days.

**2.5 Sample collection and biochemical analyses**

At the end of the experiment (14 days), the animals were subjected to an overnight fast, weighed using a weighing balance (Doran-PC 500), anesthetized by the “Drop method” (JHU, 2009) and blood samples taken from their jugular vein. Whole blood samples were draw into heparinized capillary tubes and spun immediately in a microhematocrit centrifuge (Hawksley hematospin 1400) for 5 mins at 1500g as described by Bain et al. (2008) to estimate pack cell volume (PCV). Haemoglobin estimation was carried using the Cyanmethaemoglobin method as described by Bain et al. (2008). The remaining blood samples were dispersed into plain containers labeled appropriately and allowed to coagulate. The samples were centrifuged in a Power Spin Centrifuge (C858E) for 10 minutes at 1500g within two hours after collection. The serum obtained was stored frozen until analyses. Immediately after the blood collection the rats were sacrificed and dissected. The liver was excised, blotted dry, weighed on a microbalance sensitive at 0.001mg (Precissa 125A, Switzerland) and recorded. Liver samples were fixed in 10% formal saline until histopathological analysis. Biochemical analyses were carried out to determine the serum concentrations and activity of some enzymes such as AST and ALT (using the Reitman and Frankel
2.7 Automatic tissue processing

The tissues were processed in an automatic tissue processor (Jung Histokinette 2000) as follows: Beaker 1 (containing 10% formal saline) for 3 hours, Beaker 2 (containing 70% alcohol) for 1 hour, Beaker 3 (containing 80% alcohol) for 2 hours, Beaker 4 (containing 90% alcohol) for 2 hours, Beaker 5 (containing 95% alcohol) for 2 hours, Beaker 6 (containing 95% alcohol) for 2 hours, Beaker 7 (containing absolute alcohol I) for 2 hours, Beaker 8 (containing absolute alcohol II) for 2 hours, Beaker 9 (containing xylene I) for 3 hours, Beaker 10 (containing xylene II) for 3 hours, Wax bath I for 3 hours, Wax bath II for 3 hours. After the last processing stage, tissues were covered in molten paraffin wax in metallic embedding moulds, allowed to cool and solidify in a refrigerator for 15 minutes at 5°C. The cooled and solidified tissue blocks were trimmed to remove excess wax and serially sectioned at 5 µm on a rotary microtome (KEDEE KD-3668AM). The sections were floated in 20% alcohol and transferred to a water bath (HH-420) at 45°C and picked up at an angle using clean frosted end slides. Adequate attachment of tissues to slides was ensured by placing the tissue and slide on a hot plate for a duration of 40 minutes.

2.7 Haematoxylin and Eosin staining

The tissue sections were dewaxed in 2 changes of xylene for 2 minutes each, hydrated through decreasing grades of alcohol (that is, 100%, 90%, 80% and 70%) for 2 minutes each, stained in Ehrlich's haematoxylin for 30 minutes and rinsed in running tap-water to remove excess stain. The sections were differentiated in 1% acid alcohol for a second, blued in running tap water 10 minutes, counterstained with 1% eosin for 2 minutes, rinsed in water, dehydrated in ascending grades of alcohol (70%, 80%, 95% and absolute), dealkoholized in xylene, dried at room temperature and mounted using dibutylphthalate propylene xylene. The slides were examined under a light microscope and photomicrographs were taken using a camera attached to the microscope.

2.8 Statistical Analysis

The biochemical data were subjected to some statistical analysis: analysis of variance (ANOVA), Partial correlation analysis and Post Hoc test was carried out on the data using the Statistical Package for Social Sciences (SPSS; version 18). Values were reported as Mean ± SD. P-value is significant at p<0.01 and p<0.05.

3.0 RESULTS

Table 1: Phytochemical analysis of Annona muricata

<table>
<thead>
<tr>
<th>Phytochemical components</th>
<th>Quantification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate</td>
<td>++</td>
</tr>
<tr>
<td>Proteins</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>++</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>++</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>++</td>
</tr>
</tbody>
</table>

In table 2 below, the test groups showed insignificant overall increase in the level of Hb and PCV when compared with the control group (p>0.05). However, significant increases were observed in the level of Hb and PCV when groups 2 and 4 were compared with the control group (p<0.05). Insignificant decrease was observed in group 2 while insignificant increase were observed in groups 3 and 4 when the level of ALP was compared with that of the control group (p>0.05). Generalized insignificant decreases were observed in the level of ALT when the test groups were compared with the control group (p>0.05). It also shows significant differences in the level of serum AST within and between the groups (p<0.05). However, no significant increase was observed when group 2 was compared with the control group nor any significant decrease observed when groups 3 and 4 were compared with group 1 (p>0.05). Overall insignificant decrease was observed in the activity of LDH and Amylase when the test groups were compared with the control group (p>0.05). Comparing the weight of the liver, insignificant increases were observed when the test groups were compared with the control group (p>0.05).

Table 3 above showed no significant change in body weight when the weight of the animals (in each group) before the commencement of the experiment was compared with the weight of the animals after the experiment (p>0.05). The table also shows that group two had higher change in body weight when compared with other groups. Dose dependent increase in the organ: body weight ratio (relative weight; organ weight divided by body weight after experiment multiple by 100) was observed in the tests groups when compared with the control group (p>0.05).
Figure 1 (Group 1; control) administered 2 ml of distilled water: photomicrograph of a liver section showing normal tissue microarchitecture. It shows distinct liver sinusoids (marked by green arrows), hepatocytes (marked by blue arrow heads), and central vein (marked by black arrow). H&E stained. X400

Figure 2 (group 2; administered 25 mg/kg body weight of Annona muricata): photomicrograph of a liver section showing no obvious pathological change. H&E stained. X400

Figure 3 (group 3; 50 mg/kg body weight of Annona muricata): photomicrograph of a liver section showing moderate generalized cytoplasmic necrosis. H&E stained. X400

Figure 4 (group 4; administered 100 mg/kg body weight of Annona muricata): photomicrograph of a liver section showing mild generalized cytoplasmic necrosis and stromal erosion (hepatitis). H&E stained. X400

Table 2: mean comparison of the effect of aqueous stem bark extract of Annona muricata on haematological, biochemical and organ weight based on group treatments.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Hb</th>
<th>PCV</th>
<th>ALP</th>
<th>AST</th>
<th>LDH</th>
<th>Amylase</th>
<th>Liver wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>16.40 ± 2.48</td>
<td>49.20 ± 7.40</td>
<td>492.50 ± 169.27</td>
<td>22.50 ± 11.18</td>
<td>72.00 ± 13.88</td>
<td>145.00 ± 10.41</td>
<td>220.33 ± 27.98</td>
</tr>
<tr>
<td>Group 2</td>
<td>22.74 ± 3.83</td>
<td>68.20 ± 11.52</td>
<td>597.16 ± 188.60</td>
<td>18.50 ± 5.32</td>
<td>85.33 ± 8.11</td>
<td>132.00 ± 19.42</td>
<td>192.50 ± 37.56</td>
</tr>
<tr>
<td>Group 3</td>
<td>19.12 ± 3.34</td>
<td>66.83 ± 11.03</td>
<td>501.00 ± 153.90</td>
<td>17.67 ± 3.56</td>
<td>66.83 ± 12.95</td>
<td>140.83 ± 36.91</td>
<td>179.00 ± 30.65</td>
</tr>
<tr>
<td>Group 4</td>
<td>20.75 ± 3.56</td>
<td>66.83 ± 11.03</td>
<td>501.00 ± 153.90</td>
<td>17.67 ± 3.56</td>
<td>66.83 ± 12.95</td>
<td>140.83 ± 36.91</td>
<td>179.00 ± 30.65</td>
</tr>
</tbody>
</table>

*Mean difference is significant at P<0.05. Analysis of variance and Post Hoc test, N=24, n=6

Keys: Hb= Haemoglobin, PCV= Packed Cell Volume, ALP= Alkaline Phosphatase, AST= Aspartate transaminase, ALT= Alanine transaminase, LDH= Lactate Dehydrogenase

Table 3: T-test analysis comparing animal weight before and after the experiment

<table>
<thead>
<tr>
<th>Groups</th>
<th>Wt. Before</th>
<th>Wt. After</th>
<th>Mean diff. in weight</th>
<th>Organ/ Body wt</th>
<th>P-value</th>
<th>T-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>139.00±19.62</td>
<td>149.33±21.46</td>
<td>10.33</td>
<td>5.12</td>
<td>0.421</td>
<td>0.877</td>
</tr>
<tr>
<td>2</td>
<td>132.33±18.65</td>
<td>150.33±27.22</td>
<td>18</td>
<td>5.43</td>
<td>0.207</td>
<td>1.447</td>
</tr>
<tr>
<td>3</td>
<td>131.60±26.16</td>
<td>144.40±29.27</td>
<td>12.8</td>
<td>5.52</td>
<td>0.464</td>
<td>0.809</td>
</tr>
<tr>
<td>4</td>
<td>133.66±26.39</td>
<td>136.83±25.74</td>
<td>3.17</td>
<td>5.64</td>
<td>0.871</td>
<td>0.170</td>
</tr>
</tbody>
</table>

*Mean difference is significant at P<0.05. Student t-test, N=24, n=6

Key: WT= weight, diff= difference
4.0 DISCUSSION

Annona muricata have been reported to possess many therapeutic properties ranging from antilulcer to anticancer potency. Its use in the field of alternative medicine is with some controversies. Some studies have assessed its potency at 10 mg/kg on tumours with some level of success (Sundarrao et al., 1993), while some studies reported some cytotoxicity following its administration of the seed and fruit juice. However, little or no work has been done to ascertain the effect of the stem bark extract on the entire body system. This study was carried out to determine the effects of aqueous stem bark extract of Annona muricata in relation to some hematological and biochemical parameters, body and organ weight and liver architecture in albino rats in a bid to finding its safe doses.

The result of this study shows that aqueous stem bark extract of Annona muricata contain more carbohydrate, alkaloids, flavonoids and glycosides than the leaf extract as earlier reported by Vijayameena et al.(2013). The flavonoids observed in table 1 have been shown to have antioxidant activity, free radical scavenging capacity, coronary heart disease prevention, hepatoprotective, anti-inflammatory and anticancer activities, while some flavonoids exhibit potential antiviral activities (Kumar et al., 2013). They are also known to inhibit specific enzymes, stimulate some hormones and neurotransmitters (Haysteen, 2002). Saponins are a group of naturally occurring plant glycosides which possess significant anticancer properties by cell cycle arrest and apoptosis (Man et al., 2010). Tannins possess anticancinogenic and antimutagenic potentials which is pertinent in protecting cellular oxidative damage including lipid peroxidation. They are also known to modulate immune responses and produce liver necrosis (Chung et al., 1998). The phytochemical analysis also confirms that the stem bark contains alkaloids (table 1) as reported by Tattersfield and Padma (1940) and Lannuzel et al. (2003). These alkaloids have are said to possess antitumour, diuretic, anti-inflammatory, hyponoanalgesic and antidepressant properties (Aberoumond et al., 2012) and at the same time they posses some cytotoxic potency. The lower serum levels and activity of some of the enzymes evaluated in this study may be linked to the inhibitory effect by the quantified phytochemicals components (table 1). The phytochemical components of the stem bark extract may have led to the lower serum enzyme levels by reducing the overall cellular damage associated with some metabolic processes in the liver and pancreas.

The result of the study revealed increase in body and organ weight (tables 2 and 3) at 25 mg/kg body weight of the aqueous stem bark extract Annona muricata. It could be argued that the concomitant increase is non-pathological. This is supported by the fact that there were higher values of the haematological parameters, lower serum levels of ALP and ALT, and lower LDH activity when compared with the control (table 3). The higher level of Hb and PCV observed in the test groups may be attributed to the presence of the blood forming nutritional value of Annona muricata previously reported by Champy et al. (2005). This is supported by the strong positive correlation observed between Hb and PCV (table 4). The observed higher insignificant level of AST could be physiological. Furthermore, the absence of tissue damage in figure 2 suggests that the plant extract could be beneficial to the body at 25 mg/kg body weight. However, the decrease in body weight and increase in organs: body weight ration in group 4 when compared with other groups (table 3) suggests that aqueous stem back extract of Annona muricata could be toxic at 100 mg/kg body weight. This observation is in line with the findings of Agbai and Nwanegwo (2013) who reported similar decrease in body weight following the administration of methanolic leaf extract of Annona muricata.

The insignificant increase in the body and organ weights at 50 mg/kg body weight of aqueous stem bark extract Annona muricata, could be said to be of some benefit but the moderate distortion of tissue integrity observed in figure 3 suggests otherwise. However, it could still be argued that at this dosage, the plant extract was without serious damaging effect since higher values of the haematological parameters and lower serum levels of AST, ALT and LDH activity were observed when compared with the control (table 2). The higher serum level of ALP further undermines the potency and health benefit of the plant extract at 50 and 100 mg/kg body weight. The reason for the latter increase is still unclear, yet in consonance with the reports of Agbai and Nwanegwo (2013). The inverse (negative correlation) relationship between ALP and LDH (table 4) suggests that the higher ALP may be from extrahepatic architectural changes (Kim and Wyckoff, 2001). In addition, the positive correlation observed between ALP and Amylase, and the negative correlation between

Table 4: Correlation analysis between the haematological and biochemical parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>r-value</th>
<th>p-value</th>
<th>Type of correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb vs PCV</td>
<td>1.000</td>
<td>0.000</td>
<td>Significant positive</td>
</tr>
<tr>
<td>ALP vs LDH</td>
<td>-0.461</td>
<td>0.038</td>
<td>Significant negative</td>
</tr>
<tr>
<td>ALP vs Amylase</td>
<td>0.448</td>
<td>0.047</td>
<td>Significant positive</td>
</tr>
<tr>
<td>LDH vs Amylase</td>
<td>-0.672</td>
<td>0.001</td>
<td>Significant negative</td>
</tr>
</tbody>
</table>

*Mean difference is significant at P<0.05 and P<0.01. Partial correlation analysis, N=24, n=6
LDH and amylase (table 4) following administration of the extract is suggestive of pancreatic effect.

The result of the study also showed higher values of the haematological parameters and lower levels of other biochemical parameters when group 4 was compared with the control group (table 2). Group 4 had an obvious decrease in weight, though insignificant, when compared with the rest of the groups (table 3). The observable change in liver architecture (figure 4) and higher liver weight (table 2), and higher organ: body weight ratio suggests that at 100 mg/kg body weight the plant extract could be toxic. The observed toxicity may be due to presence of high amount of tannins and alkaloids in the 50 and 100 mg/kg stem bark extracts.

5.0 CONCLUSION
The findings of the study suggest that *Annona muricata* is non-toxic at 25 mg/kg body weight. Since there are evidences of reduction in serum levels and activity of enzymes following administration, it could be argued that the stem bark extracts shows promise of alleviating liver diseases by preventing cellular oxidative damage associated with some metabolic processes. Its potency may have stemmed maximally from the acetogenin, flavonoid, alkaloid and saponin contents and minimally from tannin content of the plant extract.

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REFERENCE


