SUBCHRONIC EVALUATION OF THE ETHANOL STEM BARK EXTRACT OF *ENANTIA CHLORANTHA* IN RODENTS (BIOCHEMICAL, HAEMATOLOGICAL EVALUATION AND EFFECT ON BODY WEIGHT)

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ABSTRACT

*Enantia chlorantha* is a medicinal plant used to treat malaria and other febrile illnesses in the southern and other parts of Nigeria. **Aim of study:** To determine the biochemical and haematological properties of ethanolic extract of the stem bark of *Enantia chlorantha* in rodents in Akwa Ibom State Nigeria. **Materials and methods:** Adult albino rats weighing 120-160 g of both sexes were used. They were randomly divided into four (4) groups of six (6) animals per group. Group 1 received 10 ml/kg, (p.o.) of distilled water. Groups 2-4 were given the extract (32.40, 64.80 and 96.20 mg/kg, i.p.) respectively. The extract was given at 9 am on alternate days for 28 days while toxic manifestations and mortality were being recorded. Weight changes were noted once a week. On day 29, the animals were fasted overnight and light chloroform anaesthesia was administered and blood samples collected for haematological and biochemical investigations. Haemoglobin, haematocrit, red blood cell count, white blood cell count, mean corpuscular haemoglobin concentration and mean corpuscular volume were determined using automatic counter system (K 21, Tokyo, Japan). The biochemical parameters were determined from serum obtained after centrifugation of total blood without anticoagulant at 2500 rpm for 15 min. Standardized diagnostic kits and spectrophotometer were used for determining the following: Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase, Creatinine, Urea, Total...
cholesterol, Total and Direct bilirubin, Total protein, Albumin Na⁺, K⁺, Cl⁻ levels. An autopsy was performed during which any macroscopic abnormalities were noted. The heart, kidney, liver, spleen and lungs and ovaries were removed and weighed. Samples of these organs were fixed in 10 % neutral formalin and kept in that solution for further Histopathological examination. **Results:** RBC, MCV, Hb and PCV values were not significantly affected although there was a dose dependent increase in platelet count that was significant (p<0.01) at lower doses compared to control. **Conclusion:** The above results show that the plant is safe for use and therefore supports the traditional use of *Enantia chlorantha* stem bark in treating various febrile conditions in Akwa Ibom state Nigeria.

**KEYWORDS:** *Enantia chlorantha*, haematological, biochemical, body weight stem bark, rodents.

### 1. INTRODUCTION

The plant *Enantia chlorantha* has been used for various purposes. It is usually taken orally as an aqueous solution or in alcohol. It has been known to serve as treatment for malaria, fever, bacterial infection and stomach problems (Gill and Akinwunmi, 1986). The plant is confined to West Africa. The plant extends from Southern Nigeria to Gabon, Zaire, and Angola (Keay, 1998). It is a fair-sized forest tree usually growing in dense shade and may be recognized by the bright yellow slash and conspicuous black fruits. It is an ornamental tree that may reach a height of 30m (Vivien and Faure, 1985). There are many different varieties that grow in Nigeria and the locals have different names for the plant depending on the locality. The Ibibios call it Uno eto. It is referred to as Dokita Igbo or Osupupa by the Yoruba tribe and in edo land it is called Erenbav Bogo. The plant leaves, fruit, root or stem bark. Owing to its varying uses by locals, this study is aimed at evaluating the effect of the plant extract on haematological and biochemical parameters as well as its effect on proteins as demonstrated by changes in the body weights of the rodents used.

### 2. MATERIALS AND METHODS

#### 2.1 Plant material

The plant *Enantia chlorantha* was collected in January 2015 in Uyo the Akwa Ibom state capital. It was identified by a Taxonomist in the Department of Botany and Ecological Studies, University of Uyo Akwa Ibom State Nigeria. A voucher specimen was deposited with the herbarium of the Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo.
3.2.1 Extraction
The plant stem bark was washed and partially air dried for about 2 weeks at ambient temperature (25°C ± 1°C). It was then ground to powder using pestle and mortar. The pulverized sample was divided into two parts. One part was cold macerated in 70 % ethanol at room temperature for 72 h and then filtered. The filtrate was dried in a rotary evaporator at 40 °C. This extract is referred to as crude. The other part was successively and gradiently macerated for 72 h at room temperature in the following solvents: n-hexane, chloroform, ethyl acetate, methanol and water to obtain different fractions. The crude extract and the fractions were stored in the freezer at -4°C until required.

3.2.2 Animal Stock
Mice and rats of the Swiss albino species weighing 18-25 g and 130-180 g of both sexes respectively were used for the experiments. They were obtained from the Department of Pharmacology and Toxicology Animal House in the University of Uyo, Uyo, Nigeria. The animals were housed in standard plastic cages and fed with pelleted Feeds (Bendel Feeds), and allowed water *ad libitum*.

3.3 Methods
3.3.1 Phytochemical Screening
The phytochemical screening of the extract was done according to the methods of Odebiyi and Sofowora (1978), Harbone (1984) and Trease and Evans (1996). The following bioactive compounds were screened for their presence: saponins, tannins and alkaloids. Others were flavanoids, anthraquinones, cardiac glycosides and reducing sugars.

3.4 Determination of Median Lethal Dose (LD$_{50}$)
The method of Lorke (1983) was used to determine the LD$_{50}$ of the extract in Swiss albino mice. The extract was administered to three (3) groups of mice containing 3 mice each at a dose range of 100-1000 mg/kg, (i.p.). The animals were observed for physical signs of toxicity and the number of deaths in each group within 24 hr was recorded. The animals were fasted for 24 hr prior to the experiment but allowed access to water *ad libitum*. The LD$_{50}$ was calculated as geometric mean of the maximum dose producing 0 % mortality (A) and the minimum dose producing 100 % mortality (B). LD$_{50}$ = $\sqrt{AB}$
3.12 Evaluation of Sub-chronic Toxicity of Extract in Rats

Adult albino rats weighing 120-160 g of both sexes were used. They were randomly divided into four (4) groups of six (6) animals per group. Group 1 received 10 ml/kg, (p.o.) of distilled water. Groups 2-4 were given the extract (32.40, 64.80 and 96.20 mg/kg, i.p.) respectively. The extract was given at 9 am on alternate days for 28 days while toxic manifestations and mortality were being recorded. Weight changes were noted once a week. On day 29, the animals were fasted overnight and light chloroform anaesthesia was administered and blood samples collected for haematological and biochemical investigations. Haemoglobin, haematocrit, red blood cell count, white blood cell count, mean corpuscular haemoglobin concentration and mean corpuscular volume were determined using automatic counter system (K 21, Tokyo, Japan). The organs were harvested and submitted to the Department of Anatomy in Basic Medical Sciences for the slide preparations while the interpretations of the slides were done in the Department of Histopathology in the University of Uyo Teaching Hospital. The biochemical parameters were determined from serum obtained after centrifugation of total blood without anticoagulant at 2500 rpm for 15 min. Standardized diagnostic kits and spectrophotometer were used for determining the following: Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase, Creatinine, Urea, Total cholesterol, Total and Direct bilirubin, Total protein, Albumin Na+, K+, Cl- levels. An autopsy was performed during which any macroscopic abnormalities were noted. The heart, kidney, liver, spleen and lungs and ovaries were removed and weighed. Samples of these organs were fixed in 10 % neutral formalin and kept in that solution for further histopathological examination.

(Diallo et al., 2010; Nongporn, Wantana, Chatchai and Ruthaiwan, 2010).

3.12.1 Effect of Extract on Bleeding Time

The tail tip of albino rats (150-180 g) was used for this experiment. The tip was made sterile by swabbing with 70 % alcohol and then pricked with a sterile lancet. The blood was dabbed every 15 s until the bleeding stopped. The interval between onset of bleeding and stoppage of bleeding was considered as the bleeding time (Feldman, Zinky, and Jain, 2000).

3.12.2 Determination of the Effect of Extract on Clotting Time

The tail of the rat in 3.8.0 was used for this experiment. 1-2 drops of blood was collected from the tail on a clean glass slide. The tip of an office pin is used to streak the blood every 5
s until a clot appears. The time was noted and taken as the clotting time (Feldman et al., 2000).

3.13 Statistical Analysis
Results were expressed as multiple comparisons of mean ± SEM. Significance was determined using One-way Analysis of Variance (ANOVA) followed by Turkey-Kramer multiple comparison post test. A probability level of less than 5% was considered significant.

4. RESULTS AND DISCUSSION
4.1 Sub-chronic Toxicity
4.2 Effect of Extract on Body Weight of Rats
The effect of the extract on body weight of rats treated sub-chronically is as shown in Table 1. Dose-dependent increases in weight of the extract-treated rats were observed. The increase in body weights of the extract treated groups and control were were statistically significant (p<0.01).

4.3 Effect of Extract on Haematological Parameters of Rats
The effect of sub-chronic administration of the extract on haematological parameters of rats is as shown in Table 2. The extract did not cause any observable significant (p>0.01) effect in the red blood cell count (RBC), mean corpuscular volume (MCV), Haemoglobin (Hb) concentration, bleeding time and clotting time. However, the Packed cell volume (PCV) percentage was significantly (p<0.01) increased at the middle dose of the extract (64.80 mg/kg). The platelets count was significantly (p<0.01) increased at the lower doses of the extract (32.20 and 64.40 mg/kg) compared to control in a non-dose-dependent fashion, while the highest dose of the extract caused a significant (P<0.05) decrease in platelets count relative to control. There was no statistical difference in other haematological parameters studied.

Table 1: Effect of Sub-chronic administration of extract on body weights of rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Initial Weight (g)</th>
<th>Final Weights (g)</th>
<th>Change in Body Weight</th>
<th>% wt. Gain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.2ml</td>
<td>130.0 ± 8.65</td>
<td>139.5±6.20</td>
<td>9.50</td>
<td>7.30</td>
</tr>
<tr>
<td>Extract</td>
<td>32.40</td>
<td>126.4 ±5.14</td>
<td>140.1±4.35&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.70</td>
<td>10.80</td>
</tr>
<tr>
<td>Extract</td>
<td>64.80</td>
<td>130.2 ±8.11</td>
<td>146.5± 7.42&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.40</td>
<td>12.6</td>
</tr>
<tr>
<td>Extract</td>
<td>96.20</td>
<td>130.8 ±6.55</td>
<td>149.5±9.52&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18.70</td>
<td>14.30</td>
</tr>
</tbody>
</table>

Data are represented as mean ± SEM. (n=6).

Significant at <sup>a</sup>p<0.05, <sup>b</sup>p<0.01 When compared with control (n=6).
Table 2: Effect of extract of on haematological indices of rats after subchronic administration.

<table>
<thead>
<tr>
<th>Parameters /Dose (mg/kg)</th>
<th>RBC (\times 10^6/\mu L)</th>
<th>PCV (%)</th>
<th>MCV (fl)</th>
<th>MCH (pg)</th>
<th>MCHC (g/dl)</th>
<th>Hb (g/dl)</th>
<th>Platelets (\times 10^3/\mu L)</th>
<th>Bleeding Time(S)</th>
<th>Clotting Time(S)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.87±0.38</td>
<td>36.93±0.19</td>
<td>53.90±0.00</td>
<td>17.97±0.19</td>
<td>33.3±0.00</td>
<td>12.27±0.19</td>
<td>817.33±1.74</td>
<td>2.50±0.19</td>
<td>2.75±0.20</td>
</tr>
<tr>
<td>Extract 32.40</td>
<td>6.69±0.19</td>
<td>35.87±0.19</td>
<td>53.73±0.19</td>
<td>18.13±0.42</td>
<td>33.77±0.02</td>
<td>12.13±0.19</td>
<td>920.00±0.19</td>
<td>2.60±0.42</td>
<td>2.62±0.19</td>
</tr>
<tr>
<td>64.80</td>
<td>7.04±0.00</td>
<td>38.43±0.20</td>
<td>54.63±0.19</td>
<td>18.10±0.00</td>
<td>33.10±0.00</td>
<td>12.73±0.19</td>
<td>919.00±0.77</td>
<td>2.62±0.19</td>
<td>2.58±0.20</td>
</tr>
<tr>
<td>96.20</td>
<td>6.73±0.32</td>
<td>37.43±0.19</td>
<td>55.67±0.19</td>
<td>19.03±0.1</td>
<td>34.20±0.62</td>
<td>12.80±0.00</td>
<td>757.00±0.00</td>
<td>2.66±0.20</td>
<td>2.57±0.19</td>
</tr>
</tbody>
</table>

Data expressed as mean ± SEM.

Significant at \(^a\)p<0.05, \(^b\)p<0.01 When compared with control (n=6).

4.4 Effect Extract on Total Serum Protein and Albumin of Rats

Subchronic administration of the extract (32.20–96.20 mg/kg) caused a significant (p<0.01–0.001) dose-dependent increase in the serum total protein levels when compared to control untreated rats. However, the serum albumin levels were not significantly different (p>0.05) relative to control (Table 3).

4.5 Effect of Extract on Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT) and Alkaline Phosphatase (ALP) of rats

Subchronic administration of extract did not exert any significant (p>0.05) effect on the levels of serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT) and alkaline phosphatase (ALP) of the extract treated rats respectively. Results are shown in Table 3.

4.6 Effect of Extract on Serum Total and Conjugated Bilirubin and Total Cholesterol of Rats

Sub-chronic administration of extract to rats caused significant increases in serum total bilirubin when compared to control. This increase which was non dose-dependent was only significant (p< 0.05) with the middle dose (64.40 mg/kg) of the extract (Table 3). However, the extract caused significant (p<0.001) increases in the levels of conjugated bilirubin at the middle and highest doses of the extract, while serum total cholesterol levels of the extract treated rats were not significantly (p<0.05) affected by the extract relative to control.
Table 3: Effect of extract on biochemical parameters of rats after sub-chronic administration.

<table>
<thead>
<tr>
<th>Treatment/Extract</th>
<th>Dose mg/kg</th>
<th>Total cholesterol (mMol/L)</th>
<th>Total Bilirubin (µMol/L)</th>
<th>Conjugated Bilirubin (µMol/L)</th>
<th>Total Protein (g/L)</th>
<th>Albumin (g/L)</th>
<th>AST (mMol/L)</th>
<th>ALP (mMol/L)</th>
<th>ALT (mMol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Distilled Water</td>
<td>10ml/kg</td>
<td>2.50±0.16</td>
<td>3.01±0.21</td>
<td>2.45±0.15</td>
<td>48.00±0.94</td>
<td>46.50±0.53</td>
<td>12.5±0.05</td>
<td>26.70±0.55</td>
<td>9.55 ± 1.25</td>
</tr>
<tr>
<td>Extract 32.40</td>
<td></td>
<td>2.70±0.14</td>
<td>4.05±0.32</td>
<td>2.55±0.15</td>
<td>59.70±3.15*</td>
<td>47.01±0.51</td>
<td>12.30±3.45</td>
<td>27.70±0.50</td>
<td>9.50±1.15</td>
</tr>
<tr>
<td>Extract 64.80</td>
<td></td>
<td>2.80±0.05</td>
<td>4.35±0.30 ²</td>
<td>3.75±0.17 ²</td>
<td>62.15±1.05 ²</td>
<td>47.05±0.42</td>
<td>12.15±1.20</td>
<td>26.20±0.47</td>
<td>13.81±0.65</td>
</tr>
<tr>
<td>Extract 96.20</td>
<td></td>
<td>2.99±0.14</td>
<td>3.75±0.28</td>
<td>3.67±0.16 ²</td>
<td>58.50±1.45 ²</td>
<td>46.50±0.41</td>
<td>12.50±1.10</td>
<td>26.50±0.55</td>
<td>11.65±1.20</td>
</tr>
</tbody>
</table>

Data are represented as mean ± SEM.
Significant at *p<0.05, ²p< 0.01: ³p<0.001 when compared to control (n=6).

4.7 Effect of Extract on Some Kidney Functions
The effect of sub-chronic administration of the extract at doses of 32.40 mg/kg and 96.20 mg/kg respectively to rats did not produce any significant difference (p>0.05) between mean serum concentrations of urea, Creatinine, Na⁺, K⁺ and Cl⁻ ions on the extract treated groups relative to control (Table 4).

Table 4: Effect of extract on kidney functions.

<table>
<thead>
<tr>
<th>Extract</th>
<th>Dose mg/kg</th>
<th>Urea mMol/ l</th>
<th>Creatinine mMol/ l</th>
<th>Na⁺ mMol/ l</th>
<th>K⁺ mMol/ l</th>
<th>Cl⁻ mMol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control distilled water</td>
<td></td>
<td>3.15±0.08</td>
<td>40.73±2.90</td>
<td>129.50±4.50</td>
<td>5.30±0.50</td>
<td>48.15±1.75</td>
</tr>
<tr>
<td>Extract</td>
<td>32.40</td>
<td>3.35±0.32</td>
<td>40.73±2.90</td>
<td>134.00±6.35</td>
<td>5.30±0.38</td>
<td>46.80±4.05</td>
</tr>
<tr>
<td>Extract</td>
<td>64.80</td>
<td>3.23±0.20</td>
<td>40.15±0.80</td>
<td>134.10±4.35</td>
<td>5.34±0.55</td>
<td>48.10±5.40</td>
</tr>
<tr>
<td>Extract</td>
<td>96.20</td>
<td>3.11±0.22</td>
<td>40.10±0.78</td>
<td>133.30±4.45</td>
<td>5.33±3.04</td>
<td>48.30±2.58</td>
</tr>
</tbody>
</table>

Data expressed as mean ±SEM (n=6).
Not significant when compared with control p>0.005.

DISCUSSION
The medicinal plant Enantia chlorantha is being used by the people of southern Nigeria with good results and this study has shown that the internal environment of the animals used remained relatively devoid of toxic signs. The extract caused increase in body weights relative to control. (Joo, Cho, and Kwon, 1978); (Eteng, Etarrh, and Owu, 2003). It was reported in their separate investigations that some secondary metabolites such as saponins and alkaloids stimulate protein synthesis. It is therefore inferred that the presence of these two metabolites also present in the extract among others may in part have caused the increase in weight observed in the animals. Administration of the extract did not significantly affect the...
level of RBC, Hb and MCV. Others include MCHC, MCH, clotting and bleeding times of rats. However, the level of platelets was increased significantly at the lower doses (32.40 and 64.80 mg/kg) but a significant decrease in platelet count (thrombocytopenia) was observed with the highest dose (96.20 mg/kg). This mild reduction in platelet count is corroborated in bleeding time hence the extract is not capable of interfering with clotting mechanism in animals investigated. Thrombocytopenia is indicative of an impairment of haemostatic function and herbal remedies are cited among the causes of drug-induced thrombocytopenia (Wells, Yee, Matzke, and Posey, 1987). Drug-induced immune thrombocytopenia known to result from the adsorption of drug-antibody complexes to platelet membranes, causes massive destruction of platelets by the spleen. However, the condition is usually reversible after withdrawal of the offending drug (Wells et al., 1987).

The extract caused increase in total serum protein. This effect is manifested as increase in body weight of animals which is attributable to presence of active metabolites such as saponins and alkaloids in the extract (Joo et al., 1978); (Eteng et al., 2003). Serum proteins is largely regulated via synthesis in the liver and this reflects synthetic ability of the liver (Kouitchev, Penlap, Kounam, Essame, and Etoa, 2007). An increase in serum concentration of total serum protein resulting from treatment with the extract suggests stimulation of protein synthesis by the liver.

Although the levels of serum aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase were unaffected by the administration of the extract. It is known that an increase in the enzymatic activity of ALT, AST and ALP in the serum directly reflects a major permeability or cell rupture or injury to the liver (Benjamin, 1978). The transaminases (AST and ALT) are often used as specific markers of active hepatic injury and represent markers of hepatocellular necrosis (Davern and Scharschmidt, 2002). These liver enzymes catalyse the transfer of α-amino groups of aspartate and alanine to the α-keto group of α-ketoglutaric acid (Davern and Scharschmidt, 2002). Whereas ALT activity is primarily localised in the liver and largely specific for parenchymal diseases, AST activity is present in a wide variety of tissues including heart, skeletal muscles, kidney, brain as well as the liver (Benjamin, 1978); (Ringer and Dabich, 1979); (Nduka, 1999); (Gatsing et al., 2005). The serum levels of ALT and AST are usually increased during hepatitis or liver injury (Benjamin,1978). An increase in the level of ALT usually reflects hepatic obstruction resulting from the effect of the chemical constituents of plant extract. In hepatic obstruction,
ALP increases symmetrically with bilirubin and ALT (Klaassen and Watkins, 1999); (Vasudevan and Sreekumari, 2007). Therefore, the significant increase in total and conjugated bilirubin levels, without corresponding increases in ALT, AST, ALP, and albumin level defines a mild toxic effect of the extract in the liver that maybe associated with the moderate cellular degeneration observed in the histology of the extract treated rats. Serum albumin levels are usually reduced in chronic liver diseases, congestive heart failure and nephritis (Sclavo, 1987). This mild to non-toxic effect was also observed in the kidney as urea and creatinine levels as well as in the electrolytes concentrations were unaffected by the extract administration. The electrolytes, urea and creatinine are markers of kidney function (Jesse, 1982); (Vasudevan and Sreekumari, 2007). From this, we can conclude that the plant *Enantia chlorantha* can safely be refined and prepared for further experiments to eventually produce different forms of it for human use. The relative stability of the biochemical and haematological parameters are indicative of its relative safety margin. This work corroborates the use of the plant by natives of southern Nigeria.

**REFERENCES**


