Effects of Sub-acute Oral Administration of Ethanolic Leaf Extract of *Byrsocarpus coccineus* on Haematology and Serum Biochemical Parameters of Wistar Albino Rats.

Oladele G. M., Dagana A. J. D., Akande M. G. and Okoronkwo S.

Department of Veterinary Pharmacology and Toxicology, University of Abuja, Abuja, Nigeria.

*Accepted November, 2020 and Published December, 2020*

**ABSTRACT**

*Byrsocarpus coccineus* is an indigenous herb widely dispersed in tropical Africa and commonly known as Crimson thyme. The leaf and root of the plant is noted to possess medicinal benefits in traditional medicine of the Western part of Nigeria. The plant has been reported to possess analgesic, antiplasmodial, inflammatory, antidiarrheic and antipyretic activities. The present study was therefore aimed at determining the sub-acute toxicity of ethanolic leaf extract of *Byrsocarpus coccineus*. The ethanolic leaf extract of the plant was administered orally at 250, 500, and 1000 mg/kg to three groups A, B and C of Wistar rats respectively for 14 days while the control group D was administered orally with 3ml/kg of distilled water. Blood samples were then obtained from the anaesthetized rats for haematology and serum biochemistry. The result obtained from serum analyses showed a significant increase (p<0.05) in the levels of blood urea nitrogen and creatinine levels while the increase in alkaline phosphatase is not significant for the treated groups B and C compared with the control. It was therefore concluded that high doses of the extract may have untoward effects on the kidney and therefore, the use of the plant extract in high doses should be discouraged.

**Keywords:** *Byrsocarpus coccineus*, rats, sub-acute, toxicity, haematology, serum biochemistry,
INTRODUCTION
Herbal medicine is increasingly getting popular worldwide due to reduced cost and easy accessibility to these plants. Reports have also shown that various traditional herbal medicines are used by a majority of the people in developing countries to treat a number of diseases human and animals[1] and herbal based medications are often considered to be safe as they are natural and free from untoward effects[2].

In the last few decades, there has been an increased interest in the study of medicinal properties of the plant *Byrsocarpus coccineus* which has been reported for its medicinal influence on the life of man and animals. The shrub *Byrsocarpus coccineus* Shum and Thonn commonly known as Crimson thyme belong to the family *Connaraceae* and is a popular medicinal plant used by traditionalists in the Western part of Nigeria for the treatment of various health problems of both human and animals. *Byrsocarpus coccineus* alone or in combination with other plant materials is used by the traditionalists for the treatment of diseases such as diarrhea, jaundice, impotency, piles, rheumatism, urinary disorder, venereal diseases and also in labor during pregnancy. The analgesic[3] and anti-inflammatory[4], antidiarrheic and antipyretic properties, the anxiolytic or sedative activities[5] and antidiabetic activity[6] have been reported. Ahmadu et al.[7] identified three flavonoid glycosides from the ethanolic extract of the plant and the flavonoids are quercetin 3-O-α-arabinose (guajavarin), quercetin and quercetin 3-O-β-D-glycoside.

The increase in the popularity of herbal remedies and the limited number of scientific works on their safety with respect to the efficacy, toxicity and adverse effects related to their use as remedies are widely recognized[8]. There are growing evidences that support the toxicity of herbal medicines towards their users, though various studies of the pharmacological potential of medicinal plants have been carried out, investigating their potential toxicities are very limited[9], although it has been opined that no drug should be used clinically without its clinical trials and toxicity studies[10]. Subacute oral toxicity studies of herbal medicines are therefore essential to identify the safety and the determination of dose level that could be used subsequently. It also helps in the investigation of the therapeutic index of drugs and xenobiotics[11]. The present study was therefore aimed at determining the effects of acute administration of ethanolic leaf extract of *Byrsocarpus coccineus* in rats.

MATERIALS AND METHODS

Collection and Extraction of the Plant Material
The fresh leaves of *Byrsocarpus coccineus* were obtained from Ogbooro; a village in Saki East local government area of Oyo state in Nigeria and the confirmatory identification of the plant was done at Forestry Reserve Institute of Nigeria Ibadan. The leaves were washed with water and dried at room temperature, the dried leaves were pulverized using mortar and pestle and then stored in an air tight container ready for extraction.

The pulverized dried leaf was extracted in absolute alcohol (ethanol) using cold maceration method. 250 g of pulverized sample was dissolved in 1 litre of ethanol and allowed to stay for three days with intermittent shaking the mixture obtained was then filtered using filter paper. The filtrate obtained which is the extract was concentrated using vacuum rotary evaporator maintained at an optimum temperature of 65°C. The percentage yield of the extract was 6.34%. A 10% solution of the extract was prepared with distilled water using Tween 80 from which appropriate dosages and dose volumes for the studies were calculated.

Experimental Animals
Wistar rats of both sexes weighing between 130 to 170g were used for the study. The rats were obtained from Faculty of Veterinary Medicine University of Abuja, Abuja laboratory animal house and were housed in steel metal cages at the same animal house. The animals were allowed feed and water *ad libitum*, the feed which is growers' mash (Vital feed Nigeria Ltd) was bought from local market.

Acute Toxicity Study
The acute toxicity study was conducted using modified Lorke's method[12]. The study was performed in two phases. Twelve Wistar rats divided into four groups of 3 rats each were used in the first phase; Groups A, B, and C animals were administered orally with a single
dose of 10, 100 and 1000 mg/kg of the extract respectively and the animals were observed for 24 hours to monitor their behaviour as well as if mortality will occur. This is to possibly establish the range of doses producing any toxic effect while animals in group D which is the control group were administered with 3 ml/kg of distilled water; each rat was given a single dose.

The second phase of the study involves the use of three animals, which were distributed into three groups of one animal each. The animals were administered higher doses of 1600, 2900 and 5000 mg/kg of the extract administered orally to further determine the correct LD$_{50}$ value. The animals were observed for 24 hours for behaviour as well as mortality. The LD$_{50}$ were to be calculated using the formula:

$$LD_{50} = \sqrt{D_0 \times D_{100}}$$

Where $D_0$ is the highest dose that gave no mortality and $D_{100}$ is the lowest dose that produced mortality.

**Experimental Design**

Twenty four rats divided into 4 groups of 6 animals per group were used in this study. The first 3 groups were administered with crude extract of *Byrsocarpus coccineus* at 250, 500 and 1000 mg/kg orally for 14 days. The rats in the fourth group were administered with distilled water at 3 ml/kg also for 14 days. Blood samples were then obtained from the diethyl ether-anaesthetized rats through cardiac puncture into heparinized bottles for haematological analysis. Another blood sample was collected into a clean bottle (non-heparinized) and allowed to clot. The serum was then separated from the clot and centrifuged into a clean bottle for biochemical analysis.

**Blood Analysis**

The hemoglobin concentration was determined as described by Jain[13] using the cyanomethemoglobin method. The pack cell volume was carried out using the conventional method of filling the capillary tubes with blood as described by Schalm *et al.*[14]. Erythrocyte count was determined by the hemocytometer method as described by Jain[13]. Total leukocyte and leukocyte differential count were also determined. The erythrocyte indices were determined from values obtained from red blood cell count, hemoglobin concentration, and pack cell volume.

The serum total protein was determined by Biuret method as reported by Peter Jr. *et al.*[15]. Albumin was determined according to method of Doumas *et al.*[16] while globulin level was calculated. Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) were estimated according to the method of Reitman and Frankel,[17] in which AST was determined by monitoring the concentration of oxaloacetate hydrazine formed with 2,4-dinitrophenyl hydrazine, while ALT was also determined by monitoring the concentration of pyruvate hydrazone formed with 2,4-dinitrophenyl hydrazine. Alkaline phosphatase was determined by Randox (Colorimetric) method of Rec[18] while Bilirubin estimation was carried out according to method (Colorimetric method) of Jendrasick and Grof,[19] and Sherlock[20]. Urea was determined by the diacetylmonoximemethod[21] while serum creatinine level was estimated according to Jaffe's Reaction[22].

**Statistical Analysis**

The statistical analyses were carried out using statistical package for social sciences (SPSS-computer Science). Haematological parameters, white blood cell count with its differentials and biochemical parameters were expressed as mean ± SEM. Values in all groups were compared using the analysis of variance (ANOVA) and for all analyses, the level of statistical significance was fixed at p<0.05[23].

**RESULTS**

The first phase of acute toxicity study showed no mortality and no sign of toxicity was observed. The second phase of the study also showed no mortality, but the animals were observed with frequent urination and their fecal samples were pasty.

The haematology showed no significant difference (p>0.05) when compared with the control group as shown in table 1 and table 2 which showed total white blood cell count and its differentials.
Table 1: Haematological parameters (Mean ± SEM) of rats administered with ethanolic leaf extract of *Byrsocarpus coccineus*.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>RBCx10^6/µl</th>
<th>PCV(%)</th>
<th>HB(g/dl)</th>
<th>MCV(fl)</th>
<th>MCH(pg/cell)</th>
<th>MCHC(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>250 mg/kg</td>
<td>7.56±1.8</td>
<td>44.10±2.1</td>
<td>11.80±3.2</td>
<td>51.11±1.2</td>
<td>14.90±2.8</td>
<td>30.60±3.7</td>
</tr>
<tr>
<td>500 mg/kg</td>
<td>7.13±1.2</td>
<td>39.8±2.7</td>
<td>11.87±0.9</td>
<td>51.10±3.1</td>
<td>31.20±2.7</td>
<td>7.10±0.8</td>
</tr>
<tr>
<td>1000 mg/kg</td>
<td>6.95±2.3</td>
<td>46.70±1.8</td>
<td>11.19±1.6</td>
<td>50.31±0.7</td>
<td>15.20±0.3</td>
<td>29.91±3.3</td>
</tr>
<tr>
<td>Control</td>
<td>7.72±0.9</td>
<td>45.51±1.2</td>
<td>12.10±7.1</td>
<td>50.81±0.2</td>
<td>15.61±1.8</td>
<td>30.80±2.2</td>
</tr>
</tbody>
</table>

Table 2: Total white blood cell and differentials (Mean±SEM) of rats administered with ethanolic leaf extract of *Byrsocarpus coccineus*.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TWBCx10^3/µl</th>
<th>Neut(%)</th>
<th>Lymp(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>250 mg/kg</td>
<td>7.31±2.7</td>
<td>41.60±1.8</td>
<td>58.12±2.7</td>
</tr>
<tr>
<td>500 mg/kg</td>
<td>7.10±0.8</td>
<td>42.57±3.1</td>
<td>57.34±1.4</td>
</tr>
<tr>
<td>1000 mg/kg</td>
<td>6.96±1.8</td>
<td>39.61±2.7</td>
<td>62.13±3.2</td>
</tr>
<tr>
<td>Control</td>
<td>7.52±2.1</td>
<td>47.10±1.3</td>
<td>52.81±4.2</td>
</tr>
</tbody>
</table>

The result obtained from serum biochemical analyzes (table 3) showed a significant increase (p<0.05) in the levels of blood urea nitrogen and creatinine while the increase in alkaline phosphatase and total proteins were not significant (p<0.05) for the test groups administered with 500 and 1000 mg/kg of the extract compared to the control group as shown in table 3.

Table 3: Some serum biochemical parameters (Mean±SEM) of rats administered with ethanolic leaf extract of *Byrsocarpus coccineus*.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>AST(iu/l)</th>
<th>ALT(iu/l)</th>
<th>AlkPhos(iu/l)</th>
<th>Urea (µmol/L)</th>
<th>Creatinine (µmol/L)</th>
<th>Bilirubin (µmol/L)</th>
<th>T.Pro(g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>250mg/kg</td>
<td>38.1±1.3</td>
<td>35.2±2.3</td>
<td>133.5±12.7</td>
<td>8.5±1.6</td>
<td>71.3±5.7</td>
<td>5.9±2.1</td>
<td>45.1±1.3</td>
</tr>
<tr>
<td>500mg/kg</td>
<td>39.2±0.3</td>
<td>35.1±0.7</td>
<td>157.3±5.7</td>
<td>16.8±3.2	extsuperscript{a}</td>
<td>121.5±11.7	extsuperscript{b}</td>
<td>5.5±0.3</td>
<td>45.7±0.9</td>
</tr>
<tr>
<td>1000 mg/kg</td>
<td>36.7±1.2</td>
<td>36.8±2.1</td>
<td>152.7±4.3</td>
<td>29.3±1.4	extsuperscript{a}</td>
<td>123.4±4.8	extsuperscript{b}</td>
<td>6.2±1.7</td>
<td>57.2±3.5</td>
</tr>
<tr>
<td>Control</td>
<td>37.8±3.7</td>
<td>34.2±1.8</td>
<td>148.1±13.2</td>
<td>7.8±1.5</td>
<td>69.3±1.9</td>
<td>5.8±1.5</td>
<td>64.7±1.4</td>
</tr>
</tbody>
</table>

Superscripted items indicate significant difference at p<0.05 between the test and the control groups.
DISCUSSION

The plant *Byrsocarpus coccineus* as a medicinal plant has been used severally by the traditionalists. Its antidiarrheic, analgesic, anti-inflammatory and antioxidant [24], antipyretic, anxiolytic/sedative and antidiabetic [6] activities have been researched and the plant therefore serves as a promising therapeutic agent in the nearest future. The knowledge of its toxic effect will be of paramount importance vis-à-vis its feature use as a therapeutic agent. The haematological indices in animals are important in determining the risk of toxicity of a plant extract since the changes in the blood system have a higher predictive value for human toxicity [25], the assessment of haematological parameters can therefore be used to explain hematological functions of a chemical compound or plant extracts in an organism [26] as blood act as a pathological reflector of the status of exposed animals to toxicants and other conditions or agents [27]. The present study showed no significant increase or decrease in the haematological parameters, thereby suggesting that the plant extract has no effect on the blood production or haemolysis.

Plant extracts have been used as immunostimulants as some alkaloids, tannins, phenolic compounds and flavonoids found in plants have generally been reported to be immunostimulants [28,29]. Total white blood cell count and the differentials are indicators of the ability of an organism to eliminate infection. An increase in the number of circulating leukocytes is rarely due to an increase in all the types of leukocytes. Neutrophils attack and destroy pathogens in the blood [30] the increased neutrophil counts therefore improve the phagocytic activity in the animals. Lymphocytes are the main effector cells of the immune system; they make antibodies, prime pathogens for destruction and then make memory cells ready that can go into action at any time, remembering a previous infection with a specific pathogen. Similarly, increased levels of eosinophils and basophils may suggest positive effect on the immune system as eosinophils work by releasing toxins from their granules to kill pathogens such as parasites and worms, high eosinophil counts are also associated with allergic reaction. Basophils on the other hand have the ability to secrete anticoagulants and antibodies that have function against hypersensitivity reactions in the blood stream, they act immediately as part of the immune system's action against foreign invaders as they contain histamine which dilates the vessels to bring more immune cells to the area of injury. The monocytes have been shown to increase in cases of infection; any increase in monocytes may then be ascribed to challenges on the immune system. Platelets are the blood cells involved in Coagulation [31] coagulation of blood requires that the platelets should be in sufficient size, number and function as shown by Vijayaet al. [32] where extract of *Euphorbia hirta* is used in promoting the development of blood platelets, stopping haemorrhage and preventing further bleeding. The findings in this study unfortunately showed that the plant extract have no effect on the immunity of the animals as there is no significant difference on the total white blood cell and the differentials.

The serum enzymes, Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT) and Alkaline Phosphatase (ALP) in this study showed no significant increase or decrease at all doses administered. These findings may imply that the extract has no toxic effect on the liver. This is because increases in serum enzyme activities are proportional to the extent of tissue damage. The serum ALT and AST are useful indices for identifying inflammation and necrosis of the liver [33]. ALT has its highest concentration in the liver with kidney and skeletal muscles having lesser activity of the enzyme. It can then be said that ALT measurement is more liver specific (hepatocellular inflammatory reaction) than the AST and its activity is usually greater than AST activity at early or acute hepatocellular disease [34]. The enzyme AST on the other hand tends to be released more than the ALT in chronic liver diseases such as cirrhosis [34]. The organs such as liver, bone, placenta and intestine are clinically important sources of the plasma activity of ALP. The activity of this enzyme is increased in many clinical states, the
most important being bone and liver diseases. Serum ALP is a useful diagnostic, screening and follow up tools of cholestatic hepatobiliary lesions [35]. Cholestasis is the main, if not the only liver disease responsible for increased plasma ALP activity. Thus, a normal ALP activity, in the presence of abnormal levels of other liver function parameters, may be suggestive of liver pathology other than obstruction[33]. The result of the bilirubin level which is normal is also an indication of normal liver because elevated Bilirubin is an indication of liver cell impairment. It could also be remembered that the gradual increase if present in the serum levels of unconjugated Bilirubin may be an indication that liver function is been impaired, resulting in diminished ability of hepatocytes to conjugate bilirubin. Bilirubin is a useful index of the excretory function of the liver. In the liver, bilirubin is conjugated with glucoronic acid in a reaction catalyzed by bilirubin-UDP-glucuronyltransferase which renders it soluble and subsequently excreted into the bile. The significant increase seen in the levels of urea and creatinine may be indicative of kidney problem; although creatinine is usually a more accurate marker of kidney function than urea, the elevated levels of creatinine, urea and uric acid are positive risk of renal impairment.

**Conclusion**

It was therefore concluded that the high doses of ethanolic extract of the leaf of *Byrsocarpus coccineus* could be toxic even at sub-acute level and therefore caution should be taken when using the extract for therapeutic purposes in order to prevent untoward effects.

**Statement on Conflicts of Interest**

None declared. The study was financed by the authors with the facilities and material support by the Department of Veterinary Pharmacology and Toxicology, University of Abuja, Nigeria.

**REFERENCES**


