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Serum Haematological Response of Rats Fed with African Palmyra Palm (*Borassus aethiopum*) Shoots Obtained in Sokoto State, Nigeria

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African Palmyrah palm *(B. aethiopum)* shoots "Muruchi" is widely consumed as an important source of food for the rural people in Northern Nigeria. The study investigates changes in the hematological parameters in rats fed with diets containing varying concentration of *Borassus aethiopum* shoots. Thirty two albino rats weighing 165g to 250g of both sexes were used for this study. Standard methods were used to assess the behavioral changes and haematological analyses on the animals and the blood respectively. The results showed no any change in the normal behavior of the rats fed with the shoots when compared with the control group, however no mortality was recorded throughout the period of treatment. White blood cells (WBC), Red blood cells (RBC), Hemoglobin (Hb), Packed cell volume (PVC), Mean cell hemoglobin (MCH), Mean cell volume (MCV) and Mean cell hemoglobin concentration (MCHC) in all the treated groups showed a general decrease not significantly ($p<0.05$) different compared with the control. While platelets showed a significance ($p < 0.05$) increase at higher doses compared with the control. On the other hand, lymphocytes, monocytes, eosinophils and neutrophiles showed a general increase compared to the control group, while basophiles was not detected. The results obtained suggests that *Borassus aethiopum* shoots is relatively nontoxic and has the potential of defending the body against infection and also has hematinic and blood enhancing quality.

Keywords: *Borassus aethiopum,* shoots, Haematology, Red Blood Cell, White Blood Cell.

1. Introduction

The plant African Palmyra Palm ***(****Borassus aethiopum*) plate 1 is a genus of five species of fan palms, native to tropical regions of Africa, Asia and New Guinea (Sakande *et al*., 2011).. These massive palms can grow up to 30 m (98 ft) high and have robust trunks with distinct leaf scars; in some species the trunk develops a distinct swelling just below the crown, though for unknown reasons. The leaves are fan-shaped, 2–3 m long and with spines along the petiole margins (no spines in *B. heineanus*). The leaf sheath has a distinct cleft at its base, through which the inflorescences appear; old leaf sheaths are retained on the trunk, but fall away with time. All *Borassus* palms are dioecious, with male and female flowers on separate plants; male flowers are less than 1 cm long and in semi-circular clusters, sandwiched between leathery bracts in pendulous catkins; female flowers are 3–5 cm wide, globe-shaped and solitary, sitting directly on the surface of the inflorescence axis. The fruits are 15–25 cm wide, roughly spherical and each contain 1-3 large seeds (Bayton, 2007). Depending on species, fruit color varies from black to brown, yellow or orange; the fibrous pulp is aromatic and sweet to taste. Each seed is enclosed in a woody endocarp, which protects it when the fruit is consumed by elephants, monkeys and other frugivorous. At germination, the young seedling extends downwards into the soil and only a few leaves are visible above ground; this provides some protection against frequent fires in its savanna habitat; after an indeterminate number of years (the establishment phase), the seedling forms a stem and quickly grows above the savanna vegetation, where it is then less vulnerable to fire (Bayton, 2007).

The Palmyra tree is the official tree of Tamil Nadu. In Tamil culture it is called *karpaha*,"nungu" "celestial tree", and is highly respected because all its parts can be used. The recently germinated seeds form fleshy sprouts below the surface which can be boiled and eaten as a fibrous, nutritious food. The germinated

seed's hard shell is also cut open to take out the crunchy kernel which tastes like a water chestnut but sweeter. The ripe fibrous outer layer of the fruits is edible after boiling or roasting. When the fruit is tender, the kernel inside the hard shell is an edible jelly that is refreshing and rich in minerals. In ancient times, dried palm leaves were used to write manuscripts (Bayton, 2007).

Borassus aethiopum is found in Benin, Burkina, Cameroon, Central African Republic, Chad, Comoros, Ethiopia, Ghana, Ivory Coast, Kenya, Madagascar, Malawi, Mali, Mozambique, Niger, Nigeria, Senegal, Sudan, Tanzania, Togo, Uganda, Zaire, Zambia, and Zimbabwe (Sakande *et al*., 2011).

Plate 1: *Borassus aethiopum* tree. **Source:** http://en.m.wikipedia.org/wiki/Borassus#/media/File:Borassus_flabellifer.jpg

The shoot of *Borassus aethiopum* (plate 2) is obtained by burying the matured seeds of the plant in pit and allowed to germinate (Mazumdar, 2004). The young germinating shoot or hypocotyls known as *Muruchi* or *Gazari*, is usually harvested after 7 to 8 weeks of planting (Ahmed *et al*., 2010). *Muruchi* is an important source of food for the rural people in Northern Nigeria. The people consume it either raw or boiled and claimed that it enhances *libido* in women and *aphrodiasiac* in men (Akinniyi *et al*., 2010). The shoots are potential source of starch in Cote d'Ivoire which is an important raw material in industry (Mazumdar, 2004). Akinnnyi and Waziri (2011) reported high concentration of carbydrate (83.00%) and crude fiber (3.96%) and low fat (1.49%) in the shoot of *Borassus aethiopum* on dry weight basis. Similarly, the shoots contained an appreciable amount of both macro and micro mineral elements of which potassium (236.70mg/100g), Magnesium (640mg/100g), and calcium (433.30mg/100g) are the predominant macro elements on dry weight basis. Manganese (12.85mg/100g), Zinc (12.74mg/100g), and iron (11.51mg/100g) are the predominant micro elements (Kabiru *et al*., 2015).

Plate 2: *Borassus aethiopum* shoots *(Muruchi)* **Source:** www.hort.purdue.edu/newcrop/faminefoods/ff_families/palmae.html

Despite the nutritional benefits of *Borassus aethiopum* shoots commonly known as "*Muruchi*" in Hausa speaking language, the shoots also contain some anti-nutrients such as phytate, tannins, oxalate, hydrocyanic acid and nitrate which may hinder the bioavailability of some essential minerals present in the shoot (Kabiru *et al*., 2015).

Studies on the acute and sub-chronic toxicity of the *Borassus aethiopum* shoots indicated low level of toxicity at higher doses which become imperative to carry out hematology studies in order to ascertain its toxicity using hematological parameters (Muhammad *et al*., 2019). The present study therefore aimed at investigating serum hematological response of rats fed with *Borassus aethiopum* shoots obtained in Sokoto State, Nigeria in order to evaluate its safety as source of nutrients supplement.

2. Materials and Methods

2.1 Sample Collection and Treatment

The matured shoots of *Borassus aethiopum* plant were collected from the area of cultivation in Kware Local Government Area, Sokoto State, Nigeria. The sample was collected in black polythene bags and transported to laboratory. The seedlings were dehulled, washed with distilled water, chopped in pieces, milled and then air dried. The dried sample was then pulverized into powder using pestle and mortar, and then sieved. The powdered sample was stored in a clean polythene bag until when required for analysis.

2.2 Haematological Studies

Animals

Albino rats (females) weighing 165 to 250g were purchased from the Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria. The animals were kept at the animal

house of the department in a wire mesh cages. They were fed with poultry grower's feed and tap water *ad libitum* for two weeks to acclimatize before starting the experiment. Animal treatment and handling was done according to the ethical guidelines reported by Zimmerman (1983) and in accordance with U. S. guidelines as contained in the National Institute of Health guide for the care and use of laboratory animals (NAS, 2011).

2.3 Experimental Design

Twenty four (24) female albino rats weighing between 165 – 250 g were group into six groups (five rats per group). The animals were housed in stainless steel and fed with the following diets for 4 weeks (28 days).

Group 1: Control (100% poultry growers mash).

Group 2: fed with 75% poultry growers mash + 25% pulverized *Borassus aethiopum* shoots.

Group 3: fed with 50% poultry growers mash + 50% pulverized *Borassus aethiopum* shoots.

Group 4: fed with 25% poultry growers mash + 75% pulverized *Borassus aethiopum* shoots.

2.4 Collection of Blood and Haematological analyses

At the end of the 28th day, feed and water were withdrawn from the animals overnight and the animals were anaesthetized in a container saturated with chloroform vapor and then slaughtered. Blood samples were collected at slaughter into labelled bottles coated with ethylene diaminetetraacetic acid (EDTA) as anti-coagulants and was used for red blood cells count, white blood cells count, platelets count, hemoglobin, packed cell volume and red blood cell indices as described by Lentowsky and Ciesla, (2007).

2.5 Physical Observation and Mortality

Clinical observation was made once a day for mortality, ill health or reaction to treatment, such as changes in skin and fur, eyes and mucus membranes, behavior pattern, salivation and diarrhea.

2.6 Determination of White Blood Cell Count (WBC)

The counting of total white blood cells was done using a diluting fluid (Turks fluid) in a ratio of 1:20 and then counted with an improved Neubauer counting chamber under a light microscope (mcArthur microscope) using a x10 objective in an area of 4sqmm. The cells appeared as small black dots and the WBC was calculated using equation 1:

$$WBC = \frac{\text{cell counted x blood dilution x chamber depth}}{\text{Area of chamber counted}} \quad (1)$$

2.7 Determination of Red Blood Cell Count (RBC)

The red blood cells (RBC) count was done using the conventional method of Dacie and Lewis (2001). Blood was diluted to 1:200 with Hayem's fluid which preserved the corpuscles and then counted with an improved Neubauer counted chamber under a light microscope (Mc Arthur Microscope) using a x40 objective in an area of 1 5 sqmm. Their characteristic pink-red color was used for their identification, and the RBC was then calculated using equation 2:

$$RBC = \frac{\text{cell counted x blood dilution x chamber depth}}{\text{Area of chamber counted}} \quad (2)$$

2.8 Determination of Packed Cell Volume (PCV)

The packed cell volume (PVC) was done using the macrohematocrit method (Dacie and Lewis, 2001). The blood sample was added to a bottle containing heparin (0.1mg/ml of blood). The hematocrit tube was filled to 100mm with a capillary pipette and it was centrifuged at 3,000 rpm for 30 minutes. The height of the red blood cells was read and the results were expressed as a percentage of packed cell volume.

2.9 Determination of Platelets

The platelets were determination by diluting the blood in one percent (1%) ammonium oxalate which haemolysed the red blood cells. The platelets were then counted in a definite area using the rulings of an improved Neubaucer counting chamber. Their characteristic Mauve-pink color was used in their identification.

2.10 Estimation of Haemoglobin

The conventional method (Sahli's haemoglobinometer) was employed for the estimation of hemoglobin (Hb) content of the blood. Using the Sahli haemoglobinometer, the color of the test solution was filled to 20ml mark with 10N hydrochloric acid.0.02ml of blood was added and the content of the test tube was mixed with glass rod. It was left for 5 minutes (for the hemoglobin to be changed into acid haematin). More acid was thereafter added and the mixture was stirred until the color of the test solution matched that of the colored glass standard. The

level of the fluid in the tube was read and the hemoglobin content was expressed as a percentage.

2.11 Determination of Leucocytes (Differential White Blood Cell Count)

The differential white blood cell count (Neutrophils, Lymphocytes, monocytes, Eosinophils and Basophils) was done by making a thin film of blood on a smooth edged slide. It was allowed to dry on a bench protected from dust, ants, flies, and other insects. The blood film was fixed a covered staining jar of methyl alcohol for 3 minutes. Ten (10) ml of May Grunwald Stain (mixture of 5g of May Grunwald powder and 1 litre of methanol) and 10ml of buffered water (pH 6.8) was mixed thoroughly and the smear was covered with the dilute May Grunwald stain for 3 minutes. The stain was tipped off and replaced with diluted Giemsa's stain (5%) for 9 minutes. The stain was washed off with buffered water (P^H 6.8) and clean water was dropped on the slide which was allowed to stay for 30 seconds. The water was tipped off and the slide was allowed to dry. It was then examined microscopically (McArthur microscope) for the identification of Neutrophils (cytoplasm stained pink with small mauve granules), Eosinophils (cytoplasm stained pink with large red granules), Basophils (cytoplasm contained dark mauve-blue granules) Monocytes (cytoplasm stained dull grey-blue) while lymphocytes (cytoplasm stained blue).

2.12 Determination of Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC) and Mean Corpuscular Volume (MCV)

The mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentrated (MCHC) and mean corpuscular volume (MCV) were calculated from the values obtained from red blood cells (RBC), packed cell volume (PCV) and Hemoglobin (Hb) content. They were calculated as follows:

$$MCH = \frac{\text{Hemoglobin count}}{\text{Red blood cell count}} \times \frac{1}{10} \quad (3)$$

$$MCHC = \frac{\text{Hemoglobin count}}{\text{Packed Cell Volume (PCV)}} \times \frac{100}{1} \quad (4)$$

$$MCV = \frac{\text{Packed Cell Volume (PVC)}}{\text{Red Blood Cell Count (RBC)}} \times \frac{10}{1} \quad (5)$$

2.13 Data Analysis

The data obtained was statistically analysed using one-way analysis of variance (ANOVA) with SPSS version 10.0 statistical package and the results reported as mean ± standard deviation of the values. Significant difference between the means was determined using LSD at 5% level.

3. Results and Discussion

3.1 Results

Physical Observation and Mortality

Generally, no mortality was recorded throughout the period of treatment and the sample did not produce any grossly negative behavioural changes such as excitement, restlessness, convulsions diarrhoea or coma in the rat suggesting that Borassus aethiopum shoots is relatively nontoxic.

Hematological studies

Table 1 shows the results of hematological studies of rats fed with *Borassus aethiopum* shoots. White blood cells (WBC), Red blood cells (RBC), Hemoglobin (Hb), Packed cell volume (PVC), Mean cell hemoglobin (MCH), Mean cell volume (MCV) and Mean cell hemoglobin concentration (MCHC) in all the treated groups showed a general decrease not significantly ($p<0.05$) different compared with the control. While platelets showed a significance ($p < 0.05$) increase at higher doses compared with the control. On the other hand, lymphocytes, monocytes, eosinophils and neutrophiles showed a general increase compared to the control group, while basophiles is not detected.

Table 1: Hematological Indices of Rat Fed with *Borassus aethiopum* shoots

Parameters	0.00 (Control)	25%	50%	75%
WBC(10^9dL)	12.62 ± 0.72	12.16 ± 0.87	11.94 ± 1.42	11.90 ± 1.11
RBC(10^{12}dL)	6.51 ± 0.61	6.49 ± 0.14	6.42 ± 1.56	6.42 ± 1.56
Hb (g/L)	12.08 ± 0.28	12.00 ± 0.13	11.30 ± 1.78	11.30 ± 1.78
PCV (%)	44.75 ± 0.85	44.00± 0.78	42.00 ± 1.45	41.97 ± 3.11
MCV (fL)	68.78 ± 1.75	67.99± 2.15	65.26 ± 1.72	65.00 ± 1.89
MCH (pg)	18.56 ± 0.57	18.51±3.78	17.68 ± 0.44	17.60 ± 4.99
MCHC(g/dL)	26.98 ± 0.34	27.00 ± 3.99	27.10 ± 0.20	27.19 ± 4.77
Platelet (10^9dL)	417.25 ± 27.42	520.67± 30.87	602.60 ± 96.07*	610.45±21.43*
Lympocytes (%)	80.80 ± 4.90	79.00 ± 2.10	78.76 ± 5.99	78.47 ± 3.99
Monocytes (%)	4.50 ± 0.78	4.00 ± 1.50	3.92 ± 0.87	3.51 ± 0.80
Eosinophiles (%)	0.75 ± 0.21	0.70 ± 0.10	0.60 ± 0.12	0.51 ± 0.21
Neutrophiles (%)	14.25 ± 2.50	15.00 ± 1.50	15.30 ± 2.90	15.35 ± 1.89
Basophiles (%)	ND	ND	ND	ND

Values are mean ± standard deviation; ND = Not detected; *= Significantly different from the control ($P < 0.05$) using one-way analysis of variance (6). **WBC** = White Blood Cells; **RBC** = Red Blood Cells; **Hb** = Hemoglobin; **PCV** = Packed Cell Volume; **MCV** = Mean Cell Volume; **MCH** = Mean Cell Hemoglobin; **MCHC** = Mean Cell Hemoglobin Concentration; **fL** = Femtoliters (10^{-15}); **pg** = Pictogramme (10^{-12}).

3.2 Discussion

Hematological parameters are important indices of the physiological and pathological status for both animals and humans (Adeneye *et al*., 2006). It can also be used to determine the extent of deleterious effect of foreign compounds including plant extracts on the blood of albino rats (Odeyemi *et al*., 2009). Hematological parameters can also be used to explain blood relating functions of plant extract or its products (Ajayi *et al.,* 2005).

The result indicates that the red blood cell count (RBC) of the treated groups are lower than that of the control group, but the values are generally within the normal range of 6.76 – 9.75 $x10^{12}$ L^{-1} set for rats (The Rat Fan Club, 2010). Decreased RBC may be due to an inhibited production or by increased erythrocyte destruction. It could also be due to lower level of erythropoietin (Blair *et al*, 1990). Some researchers reported that reduction in RBC count may be due to microcytic or normocytic anemia. It has been reported that altered RBC morphology due to hemolytic anemia in humans is consistent with effects observed in laboratory animals and include increased leukocytosis, red-cell fragments, and ghost cells (Blair *et al.*, 1990b). Similarly, the hemoglobin concentration generally decreased with increase in the sample fed to the albino rats and the values are within the normal range of 11.50 – 16.10 g/L set for rats (The Rat Fan Club, 2010). Lyses of erythrocytes may lead to decreased hemoglobin content which may result to a number of pathological conditions such as increased sedimentation of erythrocytes (Hong *et al*., 1989). In another study erythropenia seem to be due to reduced hemoglobin content with hemopoiesis (Dhembare, 2013). The result suggests that the shoots may have effect on the bone marrow, kidney and hemoglobin metabolism which may lead to anemia; since the value of RBC are not seriously affected (Young and Mecie, 1997).

White blood cell (WBC) count and white cell differential count are index of cellular immunity in defending the body against infection (Aboderin and Oyetayo, 2006). The white blood cell count however cannot give a definite or specific information but the result of a differential white blood cell count (Neutrophiles, eosinophiles, Monocyte, lymphocytes and Basophiles) narrows down to give specific information about infections, toxicity allergy and immuno-suppression and poisoning (Aboderin and Oyetayo, 2006). The function of lymphocytes is primarily its involvement in a variety of immunological functions, such as immunoglobulin production and modulation of immune defense (Campbell, 1996). The alteration in lymphocytes count although is within the normal range of 65 – 85% for rats may reflects possible leukopoietic and immunodulatory effects of *Borassus aethiopum* shoots. It is possible that the shoot is composed of bioactive ingredients containing hematopoietin like principle which is responsible for hematopoietins synthesis or release from hematopoietic organs such as the kidney and liver (Palani *et al*., 2009).

Moreover, an elevation in lymphocytes at higher concentrations of the shoots might be associated

with chronic inflammation of liver and kidney of rats which may results from the phytocompounds present in the feed. Eosinophiles are responsible for allergic reactions and disorders, it increases with allergic conditions and decreases with stress and/or infection (Lewis *et al*., 2006). Neutrophiles is mainly responsible for phagocytosis of pathogenic microorganism during the first few hours after their entry into tissues (Akinmutimi, 2004). Monocytes are responsible for defense of tissues against microbial agents; It increases with bacterial infection and decreases with stress (Lewis *et al.,* 2006). Basophiles counts increase upon sensitization to an antigen (or allergen).

The white blood cell (WBC) count of the treated groups are lower than that of the control group and the values are within the normal range of 6.6 – 12.60 x 10^9 L (The Rat Fan Club, 2010). Monocytes, eosinophils and neutrophils are also within the normal range of 0 – 6%, 0 – 75%, and 0 – 25% respectively set for rats (The Rat Fan Club), while basophiles are not detected suggesting that the shoots may not have adverse effect on the body system and hence relatively safe for consumption.

Platelets count measures the number of platelets. They are important for blood clotting. Increase in platelets may be due to reactive thrombocytosis as a result of hemolysis that resulted from toxic effect of some heavy metals especially lead and cadmium which were reported present in the shoots (Kabiru *et al*., 2015). Higher doses of shoots have significant effect on the platelets of the treated rats compared to the control group and this results in the changes noticed in differential white blood cell count.

Hematocrit or Packed Cell Volume (PCV) is the percentage of red blood cells (RBC) in the whole blood. The decrease in PCV maybe from the toxic effect of lead or cadmium on the bone marrow, interference with micronutrients for red cell production, inhibition of erythropoietin and increased hemolysis of the red cells from the toxic effect of the cadmium and lead which are reported present in the shoots. Changes in PCV values have been associated with stress, impaired osmoregulation and electrolyte loss (Igbinaduwa and Aikpitanyi, 2016). This suggests that the lead and cadmium induced oxidative stress resulted in impaired osmoregulation and electrolyte loss.

Increase in MCHC and decrease in MCV and MCH indicates that *Borassus aethiopum* shoots may cause anemia which may result from hemolysis and interference with some vitamins especially vitamin B12 and folic acid but not iron. An increase in MCHC value may be due to swelling of RBC and/or disturbance of osmoregulation and reduction in erythrocytes, while a decrease in MCV and MCH values seems to be correlated with decline in RBC count (Gbakon *et al*., 2018).

4. Conclusion

The results of the study suggest that *Borassus aethiopum shoots* have the potential of defending the body against infection and also have hematinic and blood enhancing quality. Further study on histopathological toxicity study is recommended to be carried out so as to ascertain the effect of *Borassus aethiopum* shoots on the different organs of the albino rats.

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Conflict of interest

The author declare no conflict of interest.

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