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HEMATOPOIETIC PROPENSITY OF ETHANOLIC LEAF EXTRACT OF *Colocasia esculenta* LINN. IN WISTAR RATS

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Abstract – The aim of this study is to evaluate the hematopoietic propensity of ethanolic leaf extract of *Colocasia esculenta* in wistar rats. Fifteen rats used for the experiment were acclimatized for seven days were divided into three groups of five rats each. Animals in group A were administered saline solution, those in group B were administered 250 mg/kg body weight of *C. esculenta* leaf extract, while those in group C were administered 500 mg/kg body weight of *C. esculenta* leaf extract. The administration was done 12 hourly for twenty-eight days via oral route since the plant is consumed orally. At the end of the treatment, animals were fasted overnight and anaesthetized using diethyl ether. Blood samples were collected by cardiac puncture. A significant increase was observed in the PCV, Hb, RBC, MCV, WBC, lymphocyte, neutrophil and platelet counts of control animals when compared with those treated with leaf extract of *C. esculenta* at $p<0.05$. The significant increase observed in the erythrocyte parameters of animals used in this study indicates that *C. esculenta* leaves have haematopoietic properties and can be used to boost blood level especially in menstruating and pregnant women as well as anaemia patients. Its ability to significantly increase white blood cell parameters is an indication that *C. esculenta* leaves can boost the immune system and thus defend the body against xenobiotics.

Keywords – *Colocasia esculenta*, Haematopoietic Propensity, Ethanolic Leaf, Anaemia, Immune System

1. Introduction

Cocoyam is a common name for more than one tropical root crop and vegetable crop belonging to the Arum family (also known as Aroids and by the family name *Araceae*) and may refer to: Taro (*Colocasia esculenta*) - old cocoyam and Malanga (*Xanthosoma* spp.) - new cocoyam [1]. Taro is consumed as a staple crop in West Africa, particularly in Nigeria, Ghana, and Cameroon. It is generally referred to as cocoyam in Nigeria, „koko“ in Yoruba, „ede“ in Igbo and „gwamba“ in Hausa languages.

The taxonomical classification of taro is as follows [2]:

Kingdom	-	Plantae
Phylum	-	Angiosperms
Class	-	Monocots
Order	-	Alismatales
Family	-	Araceae
Genus	-	Colocasia
Species	-	*C. esculenta*

Cocoyam possesses high nutritional values when compared with others like cassava and yam, with substantial vitamins, minerals and proteins contents [3]. Cocoyam leaves cannot be eaten raw. They contain a toxin that irritates the throat if not properly cooked. This is why the leaves should be soaked in cold water for about 10 to 15 minutes before they are cooked. Cocoyam has more calories than potatoes. 100 g provides 112 calories [1]. They feature high-quality Phytonutrition profile comprising of dietary fibre, and antioxidants in addition to moderate proportions of minerals, and vitamins. Cocoyam contains high levels of Vitamin A, Vitamin C, and various other phenolic antioxidants, which help to boost the immune system and help eliminate dangerous free radicals from our system. Cocoyam also plays a vital role in digestion because it consists of a high level of dietary fibre. Cryptoxanthin, which is found in cocoyam, is said to protect against both lung and oral cancers [4].

Figure 1: Cocoyam Leaves

The presence of iron and copper in cocoyam make it an important food to prevent anaemia and boost circulation throughout the body. Both iron and copper are essential for the production of red blood cells that carry the all-important oxygen to our body‟s systems and cells. Cocoyam plays an essential role in boosting the immune system. Vitamin C is found in cocoyam roots and leaves, which helps the immune system to create white blood cells that help to defend the body from foreign pathogens and agents. Vitamin C also aids the absorption of iron which improves hematopoiesis. Additionally, Vitamin C acts as an antioxidant, which moderately prevents the development of conditions such as heart disease and cancer. Azubike et al. [5] stated the hepatoprotective properties of *C. esculenta* leaves due to saponin content. Antimicrobial and anticancer activity of leaves was identified in *C. esculenta* leaves [6]. This study is therefore aimed at evaluating the haematopoietic propensity of *C. esculenta.*

2. MATERIALS AND METHODS

2.1 Collection and Extraction of Plant Materials

Fresh and healthy leaves of cocoyam were harvested at a local farm in Kuole Area of Odo-Ona, Ibadan Nigeria and were identified by a botanist. They were thoroughly washed in running water to remove contaminants. They were air dried at room temperature in an open laboratory space until they were completely dried and milled into powder using an electric blender (Moulinex). The extraction was done using soxhlet apparatus and ethanol as the solvent according to the methods described by Airaodion et al [7]. About 25 g of the powder was packed into the thimble of the soxhlet extractor and 250 mL of methanol was added to a round bottom flask, which was attached to the soxhlet extractor and condenser on a heating mantle. The solvent was heated using the heating mantle and began to evaporate moving through the apparatus to the condenser. The condensate dripped into the reservoir housing the thimble containing the sample. Once the level of the solvent reached the siphon, it poured back into the round bottom flask and the cycle began again. The process was allowed to run for a total of 18 hours. Once the process was completed, the ethanol was evaporated in a rotary evaporator at 35 ^{o}C with a yield of 2.28 g which represents a percentage yield of 9.12%. The extract was preserved in the refrigerator for further analysis.

2.2 Animal Collection

A total of thirty (30) adult Wistar rats with body weight between 150 and 170 g were used for this study. Fifteen were used for the acute toxicity studies while the remaining fifteen were used for the experiment.

2.3. Oral Acute Toxicity Studies (LD_{50})

Oral acute toxicity study was carried out according to the method described by Miller and Tainter [8]. Fifteen rats were divided into five groups (1–5) consisting of 3 rats per group. Group A was given distilled water (10 ml/kg) while groups B, C, D and E were separately given 500, 1000, 1500, and 2000 mg/kg of cocoyam extract respectively. Treatments were administered orally by gastric intubation. The animals were observed for 24 hours post treatment for signs of toxicity and then 48 hours for possible mortality.

2.4. Experimental Design

The fifteen rats used for the experiment were acclimatized for seven (7) days during which they were fed *ad libitum* with standard feed and drinking water and were housed in clean cages placed in well-ventilated housing conditions (under humid tropical conditions) throughout the experiment. All the animals received humane care according to the criteria outlined in the „Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Science and published by the National Institute of Health. They were randomly divided into three groups of five rats each. Animals in group A were administered saline solution, those in group B were administered 250 mg/kg body weight of *C. esculenta* leaf extract, while those in group C were administered 500 mg/kg body weight of *C. esculenta* leaf extract. The administration was done 12 hourly for twenty-eight days via oral route since the plant is consumed orally. At the end of the treatment, animals were fasted overnight and anaesthetized using diethyl ether. Blood samples were collected by cardiac puncture into heparinized bottles. The blood samples were centrifuge for 10 minutes using a bench-top centrifuge (Centromix) and the supernatant plasma was then used for the determinations of the biochemical parameters.

2.5. Determination of Haematological Parameters

The red blood cells (RBC) and white blood cells (WBC) counts were determined by the improved Neubauer haemocytometer method. The haemoglobin (Hb) concentration was determined according to Jain [9], using the cyanomethaemoglobin method. The packed cell volume (PCV) was determined by the microhaematocrit method according to Dacie and Lewis [10]. Schilling method of differential lecukocyte count was used to determine the distribution of the various white blood cells [11]. Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were computed according to Jain [9].

2.6. Statistical Analysis

Data were subjected to analysis of variance using Graph Pad Prism. Results were presented as Mean ± standard deviation. One way analysis of variance (ANOVA) was used for comparison of the means followed by Tukey"s (HSD) multiple comparison test. Differences between means were considered to be significant at $p<0.05$.

3. RESULTS

3.1. Acute Toxicity Studies

Ethanolic leaf extract of *C. esculenta* was safe in rats at the tested oral doses (500–2000 mg/kg). There was no mortality within the study period. However, there were behavioral changes such as depression, reduced motor activity and ataxia.

3.2. Effect of Ethanolic Leaf Extract of *C. esculenta* on Haematological Parameters in Wistar Rats

Results of the effect of ethanolic leaf extract of *C. esculenta* on Erythrocyte Parameters and white blood cell parameters in Wistar rats are presented in tables 1 and 2 respectively.

Table 1: Effect of Ethanolic Leaf Extract of *C. esculenta* on Erythrocyte Parameters in Wistar Rats after 28 days of Administration

Parameters	Control	250 mg/kg Body Weight *C. esculenta*	500 mg/kg Body Weight *C. esculenta*
PCV (%)	37.08 3.45^{a}	45.46 2.04^{b}	49.94 5.02^{c}
Hb (g/dL)	9.41 0.47^{a}	12.32 1.11^{b}	12.88 1.22^{b}
RBC (X10^{12}/L)	6.79 0.67^{a}	9.89 0.33^{b}	13.34 1.81^{c}
MCV (FL)	54.65 6.80^{a}	56.56 6.05^{a}	53.83 2.64^{a}
MCH (pg)	14.87 1.19^{a}	10.67 1.05^{b}	12.63 1.04^{ab}
MCHC (g/dL)	25.38 2.74^{a}	29.36 1.45^{b}	34.04 2.93^{c}

Values are presented as Mean standard deviation, where n = 5. Values with different superscript along the same row are significantly different at $p<0.05$.

LEGEND: PCV = Packed Cell Volume; Hb = Haemoglobin; RBC = Red Blood Cell; MCV = Mean Corpuscular Volume; MCH = Mean Corpuscular Haemoglobin; MCHC = Mean Corpuscular Haemoglobin Concentration

Table 2: Effects of Ethanolic Leaf Extract of *C. esculenta* on White Blood Cells Parameters and Platelets in Wistar Rats after 28 days of Administration

Parameters	Control	250 mg/kg Body Weight *C. esculenta*	500 mg/kg Body Weight *C. esculenta*
WBC (X10^{9}/L)	13.02 1.96^{a}	18.38 1.24^{b}	24.11 1.93^{c}
Lymphocyte (%)	38.08 6.79^{a}	47.19 3.82^{b}	58.01 5.06^{c}
Neutrophil (%)	61.98 6.79^{a}	63.38 3.10^{ab}	69.34 3.03^{b}
Platelet (X10^{9}/L)	418.02 1.96^{a}	436.00 11.27^{b}	434.98 6.93^{b}

Values are presented as Mean standard deviation, where n = 5. Values with different superscript along the same row are significantly different at $p<0.05$. WBC = White Blood Cell

4. DISCUSSION

Anaemia increases in prevalence and severity as renal function decreases, it becomes much more common at reduced glomerular filtration rate. Depending on the severity, some of the symptoms of anaemia may include: pale skin, fatigue, weakness, loss of appetite, low haematocrit and hemoglobin in a RBC etc. Factors likely to contribute to anaemia in chronic kidney diseases include blood loss, shortened red cell life span, vitamin deficiencies, the “uremic milieu,” erythropoietin (EPO) deficiency, iron

deficiency and inflammation [12,13]. However, the typical "anaemia of chronic renal insufficiency" is a result of a decreased production of red blood cells by the bone marrow. This defect in red blood cell production is largely explained by the inability of the failing kidneys to secrete hormone erythropoietin. This hormone is a necessary stimulus for normal bone marrow to produce red blood cells. Several researchers have reported the beneficial effect of *C. esculenta* leaves but there is dearth information on its effect on haematological parameters. This study is therefore aimed at assessing the haemolytic potential of its leaves in Wistar rats.

The result of the acute toxicity studies (LD_{50}) of this study showed that *C. esculenta* leaves is not toxic to health as no mortality was recorded after 48 hours of administration. The change in behaviour observed in the animals might be an indication that consumption of *C. esculenta* leaves in high amount could lead to depression.

In this study, a significant increase was observed when the blood levels of erythrocyte parameters (packed cell volume (PCV), haemoglobin (Hb), red blood cell (RBC), Mean Corpuscular Volume (MCV)) of control animals were compared with animals treated with leaf extracts of *C. esculenta* at $p<0.05$ as presented in table 1. This is an indication that there may be increased production of red blood cells therefore, suggesting the non-toxic nature of the plant to red blood cells at this period of administration. This might be due to the high phytochemical content of *C. esculenta* leaves reported by Keshav et al. [14].

The increase in the blood levels of erythrocyte parameters observed in this study might be suggestive that *C. esculenta* leaves have possible potentials to enhance erythropoietin release from the kidneys, which is the humoral regulator of RBC production and also affect the oxygen-carrying capacity of the blood and the amount of oxygen delivered to the tissues since red blood cells and haemoglobin (Hb) are very important in transferring respiratory gases [15,16]. This may be due to the high content of iron and proteins in the plant. It is therefore possible that consumption of *C. esculenta* leaves by humans can help prevent anaemia especially in menstruating and pregnant women. It has also been reported that values of RBC and associated parameters lower than normal ranges are indicative of anemic conditions while higher values are suggestive of polycythemia [17], thus, the 28-day treatment with *C. esculenta* leaves extract may not have the potential to induce anemia nor polycythemia. This is in agreement with the findings of Airaodion et al. [18] who investigated the haematopoietic properties of ethanolic leaf and seed extracts of *Telfairia occidentalis* in Wistar rats but contradicts another study of Airaodion et al. [19] who reported a significant decrease in erythrocyte parameters when animals were treated with leaf extract of *Vernonia amygdalina*. The increase observed in the erythrocyte parameters of this study is dose dependent. This might be suggestive that relatively high dose will yield better haematopoietic effect.

The results of this study also revealed a significant difference in the white blood cells parameters and platelet of control animals when compared with those treated with leaf extracts of *C. esculenta* at $p<0.05$ as presented in table 2. White blood cells, platelet, neutrophil, and lymphocytes are used to provide useful information for diagnosis in routine clinical evaluation of the state of health of a patient. Changes in the haematological system have a higher predicative value for human toxicity [20].

The increase in WBC parameters and platelet counts may be due to the presence of anti-nutritional compounds such as Terpenoids, flavonoids, Phlobatannins and tanins in *C. esculenta* leaves [14]. It has been emphasized that the high percentage of WBC especially lymphocytes are associated with the ability of the animals to perform well under very stressful conditions [21]. This increase in the WBC and percentage lymphocyte counts suggests that the phytochemical compounds present in the extracts elicited stress responses. The effect of this plant on the total WBC count could be due to the presence of flavonoid. This compound has an anti-inflammatory property and so has vital effect on inflammatory processes of some pathological states such as bacterial infection, malaria and liver diseases [22]. This

might also imply that *C. esculenta* leaves may strengthen the immune system through many cytokines regulation.

The difference in the neutrophil count of animals treated with 250 mg/kg body weight of *C. esculenta* leaves was not significant. This probably indicates that the body‟s ability to attack and destroy invading bacteria, viruses and other injurious agents (Phagocytosis) was not affected. However, the difference became significant when animals treated with 500 mg/kg body weight of *C. esculenta* leaves were compared with the control group. This possibly suggests that at this dose, the body‟s ability to attack and destroy invading bacteria, viruses and other injurious agents (Phagocytosis) was enhanced.

The significant increase in platelet count at $p<0.05$ observed in animals treated with 250 and 500 mg/kg body weight of *C. esculenta* leaves when compared with the control group respectively may be an indication that leaf extract of *C. esculenta* has stimulates the actions of platelet activating factor (PAF) and thus the blood clotting potentials. It could also be an indication that it has the potential to stimulate thrombopoietin production [23,24]. This contradicts the findings of Airaodion et al. [18, 19] who reported a nonsignificant difference in the level of platelet count when animals were treated with extract of *Telfairia occidentalis* leaves and seed as well as *Vernonia amygdalina* leaves respectively.

5. CONCLUSION

The significant increase observed in the erythrocyte parameters of animals used in this study indicates that *C. esculenta* leaves have haematopoietic properties and can be used to boost blood level especially in menstruating and pregnant women as well as anaemia patients. Its ability to significantly increase white blood cell parameters is an indication that *C. esculenta* leaves can boost the immune system and thus defend the body against xenobiotics.

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