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Effect of Ethanolic Extract of *Corchorus olitorius* Leaves on Hematological Parameters of Wistar Rats

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ABSTRACT

The affordability of herbs over expensive pharmaceutical drugs to treat diseases among non-industrialized societies is fast becoming revolutionized. Besides, synthetic drugs have been reported to have severe side effect in comparison with vegetables and herbs. This study is aimed at investigating the effect of ethanolic extract of Corchorus olitorius leaves on hematological parameters of Wistar rats. Fresh plants of C. olitorius were harvested from the Institute of Agricultural Research and Training, Moor Plantation, Ibadan. The dried leaf-powder was extracted using soxhlet apparatus and ethanol as the solvent. The ethanol was evaporated in a rotary evaporator at 35°C with a yield of 2.17 g which represents a percentage yield of 8.68%.Ten adult male Wistar rats with body weight between 150 and 170 g were used for the study. They were divided into two groups of five rats each. Animals in group A were administered saline solution while those in group B were administered C. olitorius leaf extract. The administration was done 12 hourly for twenty-eight days at 100mg/kg body weight 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 heparin zed bottles. The result of this study suggests that C. olitorius leaves can boost the immune system and thus defend the body against xenobiotics as it significantly increased the white blood cell parameters. The nonsignificant different observed in the erythrocyte parameters of animals used in this study indicates its non-toxic nature.

Keywords: *Corchorus olitorius*, Ethanolic Leaf Extracts, Anaemia, Immune System

1. INTRODUCTION

Haematopoiesis is the formation ofblood cellular components. All cellular blood components are derived from haematopoietic stem cells [1]. It is also called hematopoiesis, haemopoiesisor hemopoiesis.In a healthy adult person, approximately 10^{11}–10^{12}new blood cells are produced daily in order to maintain steady state levels in the peripheral circulation [2, 3].After birth and throughout life haemopoiesis takes place in the bone marrow. Haemolysis on the other hand is the removal of senescent or damaged red blood cells (RBCs) from the circulation [4]. Haemolysis also occurs after transfusion of stored blood. In particular, there is increasing evidence to suggest that increasing the storage period between blood donation and transfusion results in a decrease in RBC recovery and consequently an increase in post-transfusional haemolysis [5, 6]. Haemolysis is also spelt as hemolysis. It involves the rupturing

(lysis) of red blood cells (erythrocytes) and the release of their contents (cytoplasm) into surrounding fluid (e.g. blood plasma) [7]. Hemolysis may occur in vivo or in vitro (inside or outside the body). One cause of hemolysis is the action of hemolysins, toxins that are produced by certain pathogenic bacteria or fungi. Another cause is intense physical exercise [8]. Hemolysins damage the red blood cell's cytoplasmic membrane, causing lysis and eventually cell death [9].

The affordability of herbs over expensive pharmaceutical drugs to treat diseases among non-industrialized societies is fast becoming revolutionized. In some countries, it has been integrated into the health scheme despite advances in orthodox medicine. It is believed that the natural products if utilized in the correct form and dosage are less harmful than synthetic products, which most often elicit some side effects [10].

Corchorus olitorius (malvaceae) is a plant native to both tropical and subtropical regions throughout the world with mallow leaves commonly consumed as a leafy vegetable. The leaves have been reportedly used in ethnomedical practices to treat ache and pain, dysentery, malaria, enteritis, fever, gonorrhea, pectoral pains and tumors [11]. *C. olitorius* is a green leafy vegetable popularly consumed among the Yorubas of southwestern Nigeria where it is commonly called Ewedu. Among the Igbos of southeastern, Nigeria, it is called Ahihara, while in English, the plant is known as jute mallow or bush okra. *C. olitorius* plant is not found in Nigeria only but also in other countries such as Egypt, Sudan, Malaysia, South America, and the Caribbean [12, 13, 14]. Nutritional substances; including calcium, potassium, phosphate, iron, ascorbic acid, carotene and large amount of mucilaginous polysaccharides have all been identified in the plant [15]. The phytochemical composition and its toxicity have also been investigated. Medicinally, *C. olitorius* are used as a demulcent, diuretic, purgative, bitter tonic, laxative, refrigerant, carminative and lactagogue [16]. The leaves extract has given positive results in the management of chronic cystitis and dysuria. Its reported high antibacterial activity gives credence to its use traditionally for the treatment of dysentery, fever and gonorrhea [15, 16]. Airaodion *et al.* [17] reported its ameliorative efficacy of against acute ethanol-induced oxidative stress in Wistar rats. Its hypoglycemic and hypolipidaemic effect has also been reported [18].

2. MATERIALS AND METHODS

2.1 Collection and Extraction of Plant Materials

Fresh plants of *C. olitorius* were harvested from the Institute of Agricultural Research and Training, Moor Plantation, Ibadan and were identified by a botanist. The leaves were carefully removed from the stem and washed in running water to remove contaminants. They were air dried at roomtemperature in an open laboratory space for 14days and milled into powder using an electronic blender (Moulinex). The extraction was done using soxhlet apparatus and ethanol as the solvent according to the method described by Airaodion *et al.* [19, 20]. About 25 gof the powder was packed into the thimble of the soxhlet extractor. 250 mL of ethanol was added to a round bottom flask, which was attached to the soxhlet extractor and condenser on a heating mantle 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 2.17 g which represents a percentage yield of 8.68%. The extract was preserved in the refrigerator until when needed.

2.2. Experimental Design

Ten adult male Wistar rats with body weight between 150 and 170 g were purchased from the Central Animal House, College of Medicine, University of Ibadan, Nigeria. They were housed in Imrat animal house, Ibadan. They 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 two groups of five rats each. Animals in group A were administered saline solution (control group) while those in group B were administered *C. olitorius*leaf extract. The administration was done 12 hourly for twenty-eight days at 100mg/kg body weight 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 hematological parameters.

2.3. Determination of Hematological 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 [21], using the cyanomet haemoglobin method. The packed cell volume (PCV) was determined by the microhaematocrit method according to Dacie and Lewis [22]. Schilling method of differential leukocyte count was used to determine the distribution of the various white blood cells [23]. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were computed according to the method described by Jain [21].

2.4 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 AND DISSCUSSION

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 [24]. 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

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

Parameters	Control	*C. olitorius* Extract
PCV (%)	45.16 ± 3.72^{a}	46.02 ± 2.56^{a}
Hb (g/dL)	11.27 ± 2.18^{a}	12.17 ± 1.39^{a}
RBC ($X10^{12}$/L)	11.00 ± 1.27^{a}	11.91 ± 1.02^{a}
MCV (FL)	54.65 ± 6.80^{a}	56.05 ± 4.92^{a}
MCH (pg)	14.87 ± 1.19^{a}	15.14 ± 1.22^{a}
MCHC (g/dL)	25.38 ± 2.74^{a}	27.03 ± 3.53^{a}

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 Extracts of *C. olitorius*on White Cells Parameters and Platelets after 28 days of Administration in Wistar Rats

Parameters	Control	*C. olitorius* Extract
WBC ($X10^9$/L)	15.68 ± 2.03^a	19.27 ± 1.23^b
Lymphocyte (%)	38.22 ± 2.28^a	44.26 ± 4.28^b
Neutrophil (%)	61.88 ± 3.84^a	63.00 ± 6.79^a
Platelet ($X10^9$/L)	399.48 ± 4.48^a	428.90 ± 11.27^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

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. olitorius* leaves but there is dearth information on its effect on haematological parameters. This study is therefore aimed at assessing the haematopoietic potential of its ethanolic leaf extract in Wistar rats.

No significant difference was observed when the blood levels of erythrocyte parameters (packed cell volume (PCV), haemoglobin (Hb), red blood cell (RBC), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), and Mean Corpuscular Haemoglobin Concentration (MCHC) respectively) of control animals were compared with animals treated with ethanolic leaf extract of *C. olitorius*used in this study at $p<0.05$ as presented in table 1. This is suggesting the non-toxic nature of the plant to red blood cells at this period of administration.It is also an indication that the extract had no significant effect on the production of red blood cells. This result is contrary to the report of Airaodion *et al.* [25] who reported a significant difference in erythrocyte parameters when animals were treated with ethanolic leaf and seed extracts of *Telfairia occidentalis*. It also contradicts another finding of Airaodion *et al.* [26] who reported a significant decrease in erythrocyte parameters when animals were treated with ethanolic leaf extract of *Vernoniaamygdalina* for twenty-eight days.

The nonsignificant difference in the blood levels of erythrocyte parameters observed in this study might be suggestive that *C. olitorius*leaves had no effect on the release of erythropoietin from the kidneys, which is the humoral regulator of RBC production and also 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 [27, 28].

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 [29], thus, the 28-day treatment of animals with *C. olitorius*leaf extract may not have the potential to induce anemia nor polycythemia.

The results of this study revealed a significant increase in the white blood cells parameters and platelet of control animals when compared with those treated with leaf extract of *C. olitorius* 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 [30].

The increase in WBC parameters and platelet counts may be due to the presence of anti-nutritional compounds such as saponins, flavonoids and steroid

glucosides in *C. olitorius*leaves [31]. 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 [32]. This is in agreement with the study of Airaodion *et al.* [33] reported the haematopoietic potential of ethanolic leaf extract of *Talinum triangulare* in Wistar rats. 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 glycosides. 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 [34]. This might also imply that *C. olitorius*leaves may strengthen the immune system through many cytokines regulation.

The nonsignificant difference observed in the level of neutrophil count probably indicates that the body's ability to attack and destroy invading bacteria, viruses and other injurious agents (Phagocytosis) was not compromised. This contradicts the findings of Airaodion *et al.* [25] who reported a significant difference in the level of neutrophil count when animals were treated with extract of *Telfairia occidentalis* leaves and seed.

The significant increase in platelet count at $p<0.05$ observed in this study may be an indication that leaf extract of *C. olitorius* 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 [35]. This contradicts the findings of Airaodion *et al.* [25, 26] 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 *Vernoniaamygdalina* leaves respectively.

4. CONCLUSION

The result of this study suggests that *C. olitorius* leaves can boost the immune system and thus defend the body against xenobiotics as it significantly increased the white blood cell parameters. The nonsignificant different observed in the erythrocyte parameters of animals used in this study indicates its non-toxic nature.

5. ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

6. COMPETING INTERESTS

Authors have declared that no competing interests exist.

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