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Hemato-Protective Effects of *Ocimum Gratissimum* Leaves Aqueous Extract in Adult Male Wistar Rats

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Abstract

Ocimum gratissimum belongs to the Lamiaceae family. For its medicinal and culinary usefulness, it is commonly called 'alfavaca' and is cultured in countless number of farms across village and huts in Nigeria. In this study, the hemato-protective activities of *O. gratissimum* leaf extract (aqueous) was investigated in adult male wistar rats. Thirty five (35) mature male wistar rats weighing 150 – 200 g were procured, acclimatized for (two weeks) and grouped into seven (7) of five (5) rats per group (n = 5). While group I (Normal Control) received standard rat chow and water at liberty, groups II – IV received phenylhydrazine (hemolytic anemia induced, untreated – negative control), 100 mg / kg body weight (BW) of *O. Gratissimum* aqueous leaf extract (after inducing hemolytic anemia) and 5-Fluorouracil intraperitoneally for one week (Leucocytopenia induced, untreated) respectively.

Groups V-VII were respectively induced with Leucocytopenia (fed with *O. Gratissimum* aqueous leaf extract), Thrombocytopenia (untreated) and Thrombocytopenia (extract treated).

After period of administration of test substances (two weeks), blood samples were obtained from animals (using a 2ml syringe), stored in an EDTA, and passed on for laboratory assay. Results of comparisons between group means (using ANOVA) shows a statistically significant increase ($p < 0.05$) in RBC, PCV and haemoglobin (HGB) concentration of extract treated group as against the control and untreated (but anemic) group.

Also, neutrophil and WBC counts showed a significant increase ($p < 0.05$) for extract treated than control and leucocytopenia induced (negative control II) animals; whereas, there was a significant increase in platelet count for extract treated than control and thrombocytopenia induced (untreated) rats. By implication, *O gratissimum* contains potent pharmacological ingredients that may be useful in management of hematological diseases. We recommend a study on the phytochemical ingredients in *O gratissimum* for public's awareness.

Keywords

Blood; *Ocimum gratissimum*; Anemia; Leucocytopenia

Introduction

Hematology, which is the study and the use of blood cells; red cells (erythrocytes), white cells (leucocytes), and platelets (thrombocytes) is an important aspect of science that investigates the numbers and morphology of the cellular elements of blood in the diagnosis and control of diseases [1,2]. Hematological tests are valuable in the diagnosis and investigation of the degree of blood loss in many diseases [3,4]. To better explain the relationship between blood characteristics and the environment, hematological studies are of ecological and physiological importance [5] and could therefore be useful in the selection of animals that are genetically resistant to certain diseases and environmental conditions [6].

Good measures of the physiological state of animals are hematological parameters. These parameters are related to the organ formation of the blood [7] that serves as a pathological reflector of the status of toxicants and other conditions exposed to animals. As reported by Isaac et al, (2013) [8] animals with good blood composition are likely to demonstrate good performance. While laboratory blood tests are valuable tools in the animal or human body for diagnosing any deviation from normal [9], Blood testing allows the presence of certain metabolites and other constituents in the body of animals to be investigated; it thus plays a crucial role in the physiological, nutritional and pathological status of an organism [10]. Blood analysis of its components can provide useful information for the diagnosis and prognosis of animal diseases, according to Olafedehan *et al* (2010) [11]. In relation to physiological health conditions, blood constituents change [12]. In the evaluation of animal responses to different physiological conditions, these changes are significant and may be influenced by dietary. According to Afolabi et al., changes in hematological parameters are often used to assess the different state of the body and to classify stresses due to environmental, pathological and/or nutritional factors [13]. One of such often common, nutritious substance commonly referred in Africa is the scent leaf, a local

traditional herb.

Ocimum Gratissimum (scent leaf) is a plant or shrub of the *Lamiacea* family. It is mostly found in Africa and Asian continent where majority of the plant exist [14]. Several medical uses of *O. Gratissimum* were reported by Mshana et al., (2000); one of it being described to be active against several species of fungi and bacteria [15]. Some recent findings have shown *O. Gratissimum* to be useful against gonorrheal infection, vaginitis and treatment of mental illness. Though a lot of literatures on the antibacterial and antifungal action of the plant are available, little information is known about its role in the managements of haematological disorders. Thus, this study was conducted to examine the hepato-protective (blood protecting) activities of the aqueous extract of *O. Gratissimum* leaf adult male wistar rats.

Materials and Methods

Study area

The study was conducted in the animal house of the department of Human Physiology, Faculty of Basic Medical Sciences, College of Medicine, Ambrose Alli University (AAU), Ekpoma, Edo State.

Animal procurement

A total of thirty five (35) mature male wistar rats weighing between 150 – 200 g were procured. They were first and acclimatized for (two weeks) within the animal house of the study institution (AAU), while delivering standard rat chow and clean water to them at liberty.

Study design

Study design was experimental in nature. A total of thirty five (35) adult male wistar rats were grouped into seven (7) of five (5) rats per group ($n = 5$). The animals received fixed dose of test substance (*O. Gratissimum* extract) under different blood disease conditions as follows;

1. Normal Control (Group I): Fed standard rat diets and water at liberty
2. Anaemic, Untreated (Group II) Negative Control
3. Anaemic, extract treated (Group III): Fed with 100 mg / kg body weight (BW) *O. Gratissimum* aqueous leaf extract after inducing anemia (Red blood cell disease)
4. Leucocytopenic, Untreated (Group IV) Negative Control
5. Leucocytopenic, extract treated (Group V): Fed with 100 mg / kg body weight (BW) *O. Gratissimum* aqueous leaf extract after inducing leucocytopenia (White blood cell disease)
6. Thrombocytopenic, Untreated (Group VI) Negative Control
7. Thrombocytopenic, extract treated (Group V): Fed with 100 mg / kg body weight (BW) *O. Gratissimum* aqueous leaf extract after inducing thrombocytopenia (blood platelet disease)

Ethical Consent

Ethical consent was obtained from the research and ethics committee of the college of medicine, Ambrose Alli University, Ekpoma, Edo State. Also, before actual investigation, consent forms were administered to seek participants' permission.

Identification of *O. Gratissimum*

Fresh leaves of *O. gratissimum* was obtained from local farms in Ujeme, Ekpoma not to far from the main campus, AAU. The leaves were then identified and authenticated in the Department of Botany, Ambrose Alli University, Ekpoma. Identification was necessitated to suit global best practices, a recommended practice by Margaret *et al.* (1975) on studies with medicinal plants.

Extract Preparation from *O. Gratissimum* Leaf

After obtaining fresh *O. Gratissimum* (scent) leaves, leaves were air-dried at ambient temperature ($30 \pm 2^\circ\text{C}$) for 10 days. They were thereafter pulverized with mechanical grinder, obtained fine powders were stored until further needed. About 50g of the powdered sample was then dissolved in 500 ml of distilled water (via maceration) for 48hrs in line with the procedure of Eno *et al.* (2001) [16]. Weighed samples (20 g in 10 ml distilled water) of the extract was then use to prepare a 500 mg/ml stock solution that was labelled appropriately and refrigerated at 4°C until required for use.

Determination of LD_{50}

LD_{50} for oral administration of aqueous *O. Gratissimum* leaves extract to Wistar rats as recommended by Mohammed *et al.* (2007) is 1264.9 mg/kg. Safe dose is 1/10 of LD_{50} . Therefore, less than 10% doses of (126.49 mg/kg bwt) of the LD_{50} was used in this study.

Method of administration

O. Gratissimum leaves extract was orally administered to the rats using 1ml syringe with in-tube sterile cannula. The time of administration was between 8:00am and 10:00am daily as recommended by Ejebe *et al.* (2009) [17].

Inducing and Confirming Anemia

Repetitive doses of phenylhydrazine was administered to the animals in line with the methods of Crosby and Conrad, (1960) in order to induce haemolytic anaemia [18]. Blood sample collection was carried out at various times while examining the fragility profile and osmotic resistance. Animals had their baseline values measured prior to administration of phenylhydrazine.

Inducing and confirming leukocytopenia

Intraperitoneally injection of 20mg/kg body weight of 5-Flourourasilday for 2 weeks induced leukopenia in line with the procedures of Shun *et al.*, (1996) and Jain, (1986) [19]. By this method, leukopenia was induced and confirmed (by WBC count) in animals prior to feeding with *O. Gratissimum* extract.

Induction of Thrombocytopenia Reitman and Frankel, (1957)

This was achieved by daily injection of low molecular weight Heparin subcutaneously to animals at the dose of 2000 IU/kg for a period of 10 days in accordance with the protocol spelt by Reitman and Frankel, (1957) [20]. Confirmation was followed with platelet count after 10 days of heparin administration.

Statistical Analysis

Results obtained from the study were expressed as Mean \pm SEM (Standard Error of Mean), while considering p - value less than 0.05 ($p < 0.05$) was taken to be statistically significant, a one-way analysis of variance (ANOVA) was used to determine the mean differences between multiple groups. All statistical procedure was performed with the graph pad prism (version 8.0).

Results

Parameters	Parameters					
	Group I (Control)	Group II (Anaemic, Negative Control)	Group III (Anaemic, Extract Treated)	ANOVA (p-value)	Remark	
RBC ($10^{12}/L$)	3.50 \pm 0.17 ^a	3.40 \pm 0.16 ^a	7.25 \pm 1.26 ^b	0.0402	Significant	
HGB (g/dL)	10.07 \pm 0.58 ^a	9.02 \pm 0.30 ^a	13.70 \pm 1.51 ^b	0.0021	Significant	
PCV (%)	33.00 \pm 1.61 ^a	30.60 \pm 0.21 ^b	38.32 \pm 1.46 ^c	0.0006	Significant	
MCV (fl)	92.16 \pm 2.60 ^a	90.01 \pm 2.09 ^a	93.73 \pm 2.58 ^a	0.1081	Insignificant	
MCH (pg)	30.97 \pm 1.28 ^a	28.66 \pm 1.38 ^a	30.50 \pm 1.03 ^a	0.0818	Insignificant	
MCHC (g/L)	33.65 \pm 1.14 ^a	34.81 \pm 0.71 ^a	32.68 \pm 3.67 ^a	0.1000	Insignificant	

Values represent the mean \pm SEM. Values in the same row bearing the same letter of alphabets are not significantly different from each other ($p > 0.05$).

Above table compares various red blood parameters in the administration of *O. Gratissimum* leaf extract on wistar rats. From the table. PCV, RBC and hemoglobin (HGB) concentrations increased significantly ($p < 0.05$) in extract treated than control as well as anaemic, untreated groups upon comparison; even though these parameters fell insignificantly in untreated groups as compared to control. MCV MHC and MCHC however returned a statistically insignificant ($p > 0.05$) result across groups upon comparison.

Table 1: Comparative Effects of *O. gratissimum* Leaf Extract on Red Blood Cell.

Parameters	Parameters					
	Group I (Control)	Group IV (Leukopenic, Negative Control)	Group V (Leukopenic, Extract Treated)	ANOVA (p-value)	Remark	
Basophil ($10^9/L$)	0.05±0.01 ^a	0.02±0.00 ^a	0.07±0.01 ^a	0.1002	Insignificant	
Neutrophil ($10^9/L$)	7.43±1.01 ^a	6.22±1.27 ^a	3.80±0.96 ^b	0.0041	Significant	
Eosinophil ($10^9/L$)	0.17±0.04 ^a	0.24±0.03 ^a	0.19±0.09 ^a	0.3011	Insignificant	
Monocyte ($10^9/L$)	1.03±0.37 ^a	1.67±0.77 ^a	0.37±0.11 ^a	0.2305	Insignificant	
Lymphocyte ($10^9/L$)	2.71±0.29 ^a	1.29±0.19 ^a	1.85±0.63 ^a	0.0810	Insignificant	
WBC ($10^9/L$)	5.90±1.68 ^a	9.70±2.54 ^b	10.29±1.88 ^b	0.0036	Significant	
Values represent the mean ± SEM. Values in the same row bearing the same letter of alphabets are not significantly different from each other (p > 0.05).						
Above table (table II) shows a comparative effects of <i>O. gratissimum</i> leaf extract on white blood cell (leucocytes). From the table, neutrophil and WBC counts returned a statistically significant decrease and increase in extract fed group as against control animals respectively. Whereas, monocyte, lymphocyte, basophil and eosinophil counts returned an insignificant increased (p > 0.05) with ANOVA upon comparison of extract and untreated groups with normal control						

Table 2: Comparative Effects of *O. gratissimum* Leaf Extract on White Blood Cell (Leucocytes).

Parameters	Parameters					
	Group I (Control)	Group IV (Leukopenic, Negative Control)	Group V (Leukopenic, Extract Treated)	ANOVA (p-value)	Remark	
Basophil ($10^9/L$)	0.05±0.01 ^a	0.02±0.00 ^a	0.07±0.01 ^a	0.1002	Insignificant	
Neutrophil ($10^9/L$)	7.43±1.01 ^a	6.22±1.27 ^a	3.80±0.96 ^b	0.0041	Significant	
Eosinophil ($10^9/L$)	0.17±0.04 ^a	0.24±0.03 ^a	0.19±0.09 ^a	0.3011	Insignificant	
Monocyte ($10^9/L$)	1.03±0.37 ^a	1.67±0.77 ^a	0.37±0.11 ^a	0.2305	Insignificant	
Lymphocyte ($10^9/L$)	2.71±0.29 ^a	1.29±0.19 ^a	1.85±0.63 ^a	0.0810	Insignificant	
WBC ($10^9/L$)	5.90±1.68 ^a	9.70±2.54 ^b	10.29±1.88 ^b	0.0036	Significant	
Values represent the mean ± SEM. Values in the same row bearing the same letter of alphabets are not significantly different from each other (p > 0.05).						
Above table (table II) shows a comparative effects of <i>O. gratissimum</i> leaf extract on white blood cell (leucocytes). From the table, neutrophil and WBC counts returned a statistically significant decrease and increase in extract fed group as against control animals respectively. Whereas, monocyte, lymphocyte, basophil and eosinophil counts returned an insignificant increased (p > 0.05) with ANOVA upon comparison of extract and untreated groups with normal control						

Table 3: Comparative Effects of *O. gratissimum* Leaf Extract on Blood Platelets (Thrombocytes).

Discussion

Despite decades of investigations, blood disorders persist as some of the main health challenges globally, with numerous reported high death rates and associated complications. Although few systemic ailments are diagnosable in blood, in recent times however, serious attention has been focused on the effects of herbal plants on hematological health indicators [16]. While contradictory evidences surface on the matter, it has therefore become overbearing to make efforts at clarifying these reports; justifying the need for this study. Thirty five (35) mature male wistar rats of between 150 – 200 g were procured, acclimatized for (two weeks) and grouped into seven (7) of five (5) rats per group ($n = 5$). The animals received 100 mg / kg body weight of *O. Gratissimum* leaf extract (aqueous) under different blood disease conditions, while testing against extract untreated (negative control) groups for the respective haematological diseases that were induced.

Table 1 of the result section of current study shows the comparative effects of *O. gratissimum* leaf extract on red blood cells. The table compares, using one way analysis of variance (ANOVA), different red blood parameters and health indicators in the administration of *O. Gratissimum* leaf extract on adult male wistar rats. From the table. PCV, RBC and hemoglobin (HGB) levels were noticed to have significantly increased ($p < 0.05$) in extract treated than control and untreated groups upon comparison; even though these parameters decreased insignificantly in untreated groups as compared to control. MCV MHC and MCHC however returned a statistically insignificant ($p > 0.05$) result across groups upon comparison. Theoretically, the function of the red cell and its hemoglobin is to carry oxygen from the lungs or gills to all the body tissues and to carry carbon dioxide, a waste product of metabolism, to the lungs, where it is excreted. In invertebrates, oxygen-carrying pigment is carried free in the plasma; its concentration in red cells in vertebrates, so that oxygen and carbon dioxide are exchanged as gases, is more efficient and represents an important evolutionary development. In current study though, since administration of *O. Gratissimum* leaf extract significantly increased RBC, PCV and HGB levels, it suggests that *O. Gratissimum* extract is a potent agent for treating hematological conditions of low RBC such as malaria, or even haemolytic anaemia. This observation strongly conforms to previous reports of Ofem et al., (2012) [16].

Again, Table 2 shows the comparative changes in WBCs for extract treated, Leucocytopenic induced wistar rats as against the control and untreated animals. From the table, one could observe that average values of basophil count increased in extract treated than untreated and control groups, although insignificantly ($p > 0.05$). This change was however noticed to decrease significantly for neutrophil counts in extract treated than untreated and control groups; implicative that *O. Gratissimum* leaf extract (aqueous) has the capability of decreasing Neutrophil activity in immune reactions. Functionally, Neutrophils are a type of white blood cell (WBC or granulocyte) that protect from infections, among other functions. They make up approximately 40% to 60% of the white blood cells in our bodies [21], and are the first cells to arrive on the scene when one experiences an infection. A normal (absolute) neutrophil count is between 2500 and 7500 neutrophils per microliter of blood [22], and may be high with infections, due to increased production in the bone marrow as with leukemia, or due to physical or emotional stress. A low number of neutrophils may also be a sign of disease in conditions such as

leukemia, some infections, vitamin B12 deficiency, chemotherapy, and more.

In another development, the comparative effects of *O. gratissimum* leaf extract on blood platelets (thrombocytes) is shown in table III. The table reveals that administration of test substance (*O. gratissimum*) on thrombocytopenia induced rats caused a statistically significant increase ($p < 0.05$) in platelet levels of extract fed group as compared with control and non-treated animals. Since high platelet levels are implicated in increased blood clotting and thrombogenesis, the implication of this finding therefore is that *O. gratissimum* extract is a potent accelerator of blood clotting in experimental animals as seen in this table. One explanation for this could be traceable to the active ingredients in *O. gratissimum*, and the mechanism by which this is made possible; intrinsic or extrinsic, even though this is speculative. Also observed for extract treated group was thrombocytosis, which is an indicator that the extract also contains some thrombopoietin releasing compounds [23]. It is noteworthy to stress that platelets play an important role in the maintenance of homeostasis (blood clotting), which in turn is an indicator of proper platelet function, including platelet aggregation; release of thromboxane A₂, platelet factor 4, and beta- thromboglobulin; and expression of glycogen 1b and glycogen IIb / IIIa receptors [24, 25], though many studies have shown that increased platelet levels is a major risk factor for development of myocardial infarction, cerebral ischemia/transient ischemic attacks, and chronic vascular disease [26,27].

Conclusion

In this study, oral administration of *O gratissimum* notably caused an increase in RBC, PCV, hemoglobin level, Neutrophil, platelet and WBC counts, specifically significantly in extract treatments than untreated and control animals. All other haematological parameters assayed showed no significant change in *O gratissimum* administration as compared with normal rats. By implication, *O gratissimum* could contain potent pharmacological ingredients that may be useful in management of haematological diseases.

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