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

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1. Abstract

The Lamiaceae family includes Ocimum gratissimum. It's known as 'alfavaca' because of its medicinal and culinary properties, and it's grown in countless farms throughout Nigeria's villages and huts. The hemato-protective effects of O. gratissimum leaf extract (aqueous) were examined in adult male wistar rats in this study. Thirty-five (35) adult male wistar rats weighing 150–200 g were obtained, acclimatized for two weeks, and then divided into seven (7) groups of five (5) rats each (n = 5). Groups II–IV received phenylhydrazine (hemolytic anemia induced, untreated – negative control), 100 mg/kg body weight (BW) of O. Gratissimum aqueous crude extract (after generating hemolytic anemia), and 5-Flourourasilday intraperitoneal injection for one week (Leucocytopenia induced, untreated), respectively. Leucocytopenia (fed with O. Gratissimum aqueous leaf extract), Thrombocytopenia (untreated), and Thrombocytopenia (treated) were elicited in groups V-VII, respectively (extract treated). Blood samples were taken from animals (using a 2ml syringe) after two weeks of test chemical administration, preserved in EDTA, and sent to the lab for analysis. The results of group mean comparisons (using ANOVA) demonstrate that the extract treated group had a statistically significant increase (p 0.05) in RBC, PCV, and haemoglobin (HGB) concentration when compared to the control and untreated (but anemic) groups. Furthermore, neutrophil and WBC counts increased significantly (p 0.05) in extract-treated rats compared to control and leucocytopenia-induced (negative control II) rats, whereas platelet count increased significantly (p 0.05) in extract-treated rats compared to control and thrombocytopenia-induced (untreated) rats. O gratissimum, thus, includes powerful pharmacological components that could be beneficial in the treatment of hematological illnesses. For public awareness, we advocate a study on the phytochemical components of O gratissimum.

2. Introduction

Hematology, or the study and use of blood cells such as red cells (erythrocytes), white cells (leucocytes), and platelets (thrombocytes), is a branch of medicine that studies the quantities and morphology of blood's biological elements in the diagnosis and treatment of disorders [1, 2]. Hematological tests are helpful in diagnosing and determining the extent of blood loss in a variety of disorders [3, 4]. Hematological investigations are important for better understanding the relationship between blood properties and the environment [5] and could be useful in the selection of animals that are genetically resistant to particular diseases and environmental circumstances [6].

Hematological markers are good indicators of an animal's physiological status. These variables are linked to blood organ formation [7], which serves as a pathological reflector of the status of toxicants and other circumstances that animals are subjected to. According to Isaac et al. (2013) [8,] animals with healthy blood composition are more likely to perform well.

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]. 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. According to Olafedehan et al (2010) [11], blood analysis of its components can provide significant information for the diagnosis and prognosis of animal disorders. Blood constituents fluctuate in response to physiological health conditions [12]. These alterations are significant in the evaluation of animal responses to various physiological situations, and they may be altered by dietary factors. Changes in hematological parameters are frequently used to analyze the different states of the body and to identify stresses caused by environmental, pathogenic, and/or nutritional causes, according to Afolabi et al. [13]. The smell leaf, a native traditional herb, is one such common, nourishing material widely referred to in Africa.

The Lamiaceae family's Ocimum Gratissimum (scent leaf) is a plant or shrub. It is typically found in Asia and Africa, where the plant is found in abundance [14].

Mshana et al., (2000) found numerous medical uses for O. Gratissimum, one of which was described as active against several types of fungus and bacteria [15]. According to some recent discoveries, O. Gratissimum can help with gonorrhea, vaginitis, and mental disorder treatment. Although there is a wealth of literature on the plant's antibacterial and antifungal properties, nothing is known regarding its usefulness in the treatment of haematological problems. The goal of this study was to see if the aqueous extract of O. Gratissimum leaves adult male wistar rats has hepato-protective (blood-protective) properties.

3. Materials and Methods

3.1. Study Area

The experiment was carried out in the animal house of Ambrose Alli University (AAU), Ekpoma, Edo State's Department of Human Physiology, Faculty of Basic Medical Sciences, College of Medicine.

3.2. Animal Procurement

A total of 35 mature male wistar rats, weighing between 150 and 200 grams, were obtained. They were first housed and acclimatized for two weeks in the study institution's (AAU) animal house, with regular rat food and clean water delivered to them at will.

3.3. Study Design

The study's design was exploratory in nature. A total of 35 mature male wistar rats were divided into seven (7) groups, each with five (5) rats (n = 5). The animals were given a fixed dose of the test material (O. Gratissimum extract) under the following conditions:

- Normal Control (Group I): Fed standard rat diets and water at liberty
- Anaemic, Untreated (Group II) Negative Control
- 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)
- Leucocytopenic, Untreated (Group IV) Negative Control
- 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)
- Thrombocytopenic, Untreated (Group VI) Negative Control
- Thrombocytopenic, extract treated (Group V): Fed with 100 mg / kg body weight (BW) O. Gratissimum aqueous leaf extract after inducing thrombocytopenia (blood platelet disease)

3.4. Ethical Consent

The research and ethics committee of Ambrose Alli University's college of medicine in Ekpoma, Edo State, provided ethical approval. Also, prior to the inquiry, consent papers were given to the participants to obtain their permission.

4. Identification of O. Gratissimum

Fresh O. gratissimum leaves were obtained from local farms in Ujeme, Ekpoma, which is not far from AAU's main campus. The leaves were then identified and authenticated at Ambrose Alli University's Department of Botany in Ekpoma. Margaret et al. (1975) on studies using medicinal plants urged that identification be done in order to comply with global best practices.

4.1. Extract Preparation from O. Gratissimum Leaf

Fresh O. Gratissimum (scent) leaves were obtained and air-dried for 10 days at room temperature (30 2°C). After that, they were pulverized with a mechanical grinder, and the fine powders were stored until they were needed again. Following the process of Eno et al. (2001) [16], around 50g of the powdered material was dissolved in 500 ml of distilled water (through maceration) for 48 hours. Weighed extract samples (20 g in 10 ml distilled water) were then used to make a 500 mg/ml stock solution, which was labeled suitably and stored at 4CO until needed.

4.2. Determination of Ld50

According to Mohammed et al. (2007), the LD50 for oral administration of aqueous O. Gratissimum leaves extract to Wistar rats is 1264.9 mg/kg. 1/10 of the LD50 is considered a safe dose. As a result, dosages of less than 10% of the LD50 (126.49 mg/kg bwt) were employed in this study.

5. Method of Administration

The rats were given O. Gratissimum leaves extract orally using a 1ml syringe with a sterile cannula in the tube. According to Ejebe et al. (2009) [17], the administration time should be between 8:00

and 10:00 a.m. on a regular basis.

5.1. Inducing and Confirming Anemia

In order to produce haemolytic anaemia, the animals were given repeated doses of phenylhydrazine, as described by Crosby and Conrad (1960) [18]. While investigating the fragility profile and osmotic resistance, blood samples were collected at various intervals. Prior to the administration of phenylhydrazine, the animals' baseline values were assessed.

5.2. Inducing and Confirming Leukocytopenia

Injecting 20mg/kg body weight of 5-Flourourasilday intraperitoneally for 2 weeks caused leukopenia, as described by Shun et al., (1996) and Jain, (1986) [19]. Leukopenia was induced and confirmed (by WBC count) in rats using this manner before they were fed O. Gratissimum extract.

5.3. Induction of Thrombocytopenia Reitman and Frankel, (1957)

This was accomplished by injecting low molecular weight heparin subcutaneously into rats at a rate of 2000 IU/kg every day for ten days, as described by Reitman and Frankel (1957) [20]. After 10 days of heparin therapy, a platelet count was used to confirm the diagnosis.

6. Statistical Analysis

The study's findings were expressed as Mean SEM (Standard Error of Mean), and a one-way analysis of variance (ANOVA) was used to assess the mean differences across various groups, with a p-value less than 0.05 (p 0.05) being statistically significant. The graph pad prism was used for all statistical procedures (version 8.0)

7. Results

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

Parameters	Parameters				
	Group I	Group II	Group III	ANOVA	Remark
	(Control)	(Anaemic, Negative Control)	(Anaemic, Extract Treated)	(p-value)	
RBC (10^{12}/L)	3.50±0.17 [a]	3.40±0.16 [a]	7.25±1.26 [b]	0.0402	Significant
HGB (g/dL)	10.07±0.58 [a]	9.02±0.30 [a]	13.70±1.51 [b]	0.0021	Significant
PCV (%)	33.00±1.61 [a]	30.60±0.21 [b]	38.32±1.46 [c]	0.0006	Significant
MCV (fl)	92.16±2.60 [a]	90.01±2.09 [a]	93.73±2.58 [a]	0.1081	Insignificant
MCH (pg)	30.97±1.28 [a]	28.66±1.38 [a]	30.50±1.03 [a]	0.0818	Insignificant
MCHC (g/L)	33.65±1.14 [a]	34.81±0.71 [a]	32.68±3.67 [a]	0.1	Insignificant

The values show the mean minus the standard error of the mean. The values in the same row that start with the same letter of the alphabet are not substantially different ($p > 0.05$).

The table above compares several red blood parameters after wistar rats were given O. Gratissimum leaf extract. From the dining room table. When extract treated groups were compared to control and anemic, untreated groups, PCV, RBC, and hemoglobin (HGB) contents increased considerably (p 0.05), despite the fact that these parameters declined insignificantly less untreated groups when compared to control. When MCV, MHC, and MCHC were compared between groups, the results were statistically insignificant ($p > 0.05$).

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

Parameters	Parameters				
	Group I	Group IV	Group V	ANOVA	Remark
	(Control)	(Leukopenic, Negative Control)	(Leukopenic, Extract Treated)	(p-value)	
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]	0.081	Insignificant
WBC (10^9/L)	5.90±1.68 [a]	9.70±2.54 [b]	10.29±1.88 [b]	0.0036	Significant

The values show the mean minus the standard error of the mean. The values in the same row that start with the same letter of the alphabet are not substantially different ($p > 0.05$).

The effects of O. gratissimum leaf extract on white blood cells are compared in the table above (table II) (leucocytes). In comparison to control animals, neutrophil and WBC levels in the extract fed group showed a statistically significant decrease and rise, respectively. When the extract and untreated groups were compared to the normal control group, monocyte, lymphocyte, basophil, and eosinophil counts showed no significant increase ($p > 0.05$).

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

Parameters	Parameters				
	Group I (Control)	Group VI (Thrombocytopenic, Negative Control)	Group VII (Thrombocytopenic, Extract Treated)	ANOVA (p-value)	Remark
PLT (10^9/L)	123.01±5.03 [a]	87.32±3.12 [b]	219.21±9.09 [c]	0.0104	Significant

The values show the mean minus the standard error of the mean. The values in the same row that start with the same letter of the alphabet are not substantially different ($p > 0.05$).

The platelet count of thrombocytopenia-induced, extract-fed rats is presented in table III. When compared to control and non-treated rats, ANOVA revealed a significant increase (p 0.05) in platelet counts in the extract fed group.

8. Discussion

Despite decades of research, blood diseases remain one of the world's most serious health problems, with countless reports of high death rates and consequences. Although few systemic diseases can be diagnosed through blood tests, the effects of herbal plants on hematological health markers have recently received a lot of attention [16]. While conflicting evidence emerges on the subject, efforts to clarify these reports have grown excessive, necessitating the need for this investigation. Thirty-five (35) mature male wistar rats weighing between 150 and 200 grams were obtained, acclimated for two weeks, and divided into seven (7) groups of five (5) rats each (n = 5). Under distinct blood illness settings, the animals were given 100 mg/kg body weight of O. Gratissimum leaf extract (aqueous) while being tested against extract-untreated (negative control) groups for the individual haematological diseases that were produced.

The comparative effects of O. gratissimum leaf extract on red blood cells are shown in Table I of the current study's results section. The table analyzes several red blood parameters and health indicators in the treatment of O. Gratissimum leaf extract on adult male wistar rats using one-way analysis of variance (ANOVA). From the dining room table. PCV, RBC, and hemoglobin (HGB) levels were shown to be considerably higher (p 0.05) in extract-treated groups when compared to control and untreated groups, despite the fact that these parameters dropped insignificantly not untreated groups when compared to control. When MCV, MHC, and MCHC were compared between groups, the results were statistically insignificant ($p > 0.05$). The red cell and its hemoglobin purpose, in theory, is to transport oxygen from the lungs or gills to all body tissues and to transport carbon dioxide, a waste product of metabolism, to the lungs, where it is eliminated. Invertebrates have oxygen-carrying pigment that is free in the plasma; in vertebrates, it is concentrated in red cells, allowing oxygen and carbon dioxide to be exchanged as gases, which is a significant evolutionary development. However, as O. Gratissimum leaf extract significantly improved RBC, PCV, and HGB levels in the current study, it shows that O. Gratissimum extract is an effective drug for treating hematological illnesses with low RBC, such as malaria or even haemolytic anemia. This finding is in line with recent findings by Ofem et al., (2012) [16].

Table II compares the differences in WBCs between extract-treated, Leucocytopenic induced wistar rats and control and untreated animals. The table shows that average basophil count increased in the extract treated group compared to the untreated and control groups, but insignificantly ($p > 0.05$). However, neutrophil counts in extract-treated groups were significantly lower than in untreated and control groups, implying that O. Gratissimum leaf extract (aqueous) has the ability to reduce Neutrophil activity in immunological reactions. Neutrophils are a type of white blood cell (WBC or granulocyte) that, among other things, protects against infections. They make up about 40 percent to 60 percent of white blood cells in our body [21], and they are the first cells to appear on the scene when an infection occurs. A normal (absolute) neutrophil count is between 2500 and 7500 neutrophils per microliter of blood [22], and it might be high due to illnesses, increased bone marrow production (as in leukemia), or physical or emotional stress. In illnesses including leukemia, certain infections, vitamin B12 deficiency, chemotherapy, and more, a low number of neutrophils can be a symptom of sickness.

In a related development, table III compares the effects of O. gratissimum leaf extract on blood platelets (thrombocytes). The table shows that giving the test ingredient (O. gratissimum) to thrombocytopenia-induced rats resulted in a statistically significant (p 0.05) increase in platelet counts in the extract-fed group as compared to control and non-treated animals. Because high platelet levels are linked to accelerated blood clotting and thrombogenesis, this discovery implies that O. gratissimum extract is a potent blood clotting accelerator in experimental animals, as shown in the table. Even though this is theoretical, one explanation for this could be traced to the active substances in O. gratissimum and the process by which they are made possible; intrinsic or extrinsic. Thrombocytosis was also seen in the extract-treated group, indicating that the extract includes thrombopoietin-releasing chemicals [23]. Platelets play an important role in maintaining homeostasis (blood clotting), which 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 aggregation is associated with increased platelet function [24, 25].

9. Conclusion

In this study, oral administration of O gratissimum resulted in a substantial rise in RBC, PCV, hemoglobin level, Neutrophil, platelet, and WBC counts in extract treatments compared to untreated and control animals. In comparison to normal rats, all other haematological parameters were unaffected by O gratissimum delivery. O gratissimum, therefore, could include significant pharmacological components that could aid in the treatment of haematological illnesses.

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