Original Article

Effect of aqueous leaves extract of *Ocimum gratissimum* on hematological parameters in rats

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Abstract

Objective: This study aims to elucidate the effect of *Ocimum gratissimum* on hematological parameters in rats. **Materials and Methods**: Thirty male albino Wistar rats were randomly assigned into three groups of ten rats each. Group 1 was control, while groups 2 (LD) and 3 (HD) received 500 mg/kg and 1000 mg/kg body weight, respectively, of the extract orally once daily. Rats in all three groups received normal rat chow and drinking water ad *libitum* for 28 days. Complete blood count was done using an automatic counter. **Results**: The HD group had significantly ($P<.05$) higher red blood cell (RBC) counts, packed cell volume (PCV), hemoglobin (Hb), and platelet counts as compared with the control and LD groups. No significant changes were observed in the total white blood cell (WBC) count of the three groups, but significantly ($P<.05$) lower lymphocyte and higher neutrophil counts were observed in the HD group compared with the LD group. The mean platelet volume (MPV), platelet–large cell ratio (P–LCR), and platelet distribution width (PDW) were significantly ($P<.05$) reduced in the HD compared with the LD group. The mean corpuscular volume (MCV) and RBC distribution width–standard deviation were significantly ($P<.05$) lower in the HD group than in control. No significant changes were observed in levels of mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and RBC distribution width–coefficient of variation among the groups. **Conclusion:** We conclude that oral administration of O gratissimum increases RBC, PCV, Hb, platelet count, and neutrophils and also leads to a decrease in platelet indices (i.e., MPV, P–LCR, and PDW).

Key words: Blood indices, differential white blood cell, Hb, *O gratissimum*, packed cell volume, platelet, red blood cell, total WBC

Introduction

Ocimum gratissimum belongs to the family Lamiaceae. It is commonly called 'alfavaca' and is cultivated in many gardens around village huts in Nigeria for its medicinal and culinary uses.[1]

It is believed to have originated in Central Africa and South East Asia.[2] Phytochemical screening of this plant has revealed the presence of many active ingredients, such as flavonoids, triterpenes, alkaloids, citral, saponins, eugenol, linaol, methyl cinnamate, camphor, and thymol.[3,4] Eugenol, an isolate from *O gratissimum* has been observed to possess antihelminthic, nematicidal, and insecticidal properties.[5–7]

Several species and varieties of the genus *Ocimum* have been reported to yield oils of diverse nature; these are commonly called basilica oils. According to the literature, the oils produced from *O gratissimum* are active against several bacteria (including *Staphylococcus aureus, Listeria monocytogenes, Escherichia coli,* etc.) and fungi (including *Trichophyton rubum*, *T mentagrophytes*, etc.).[8–12] The oils are used in the treatment of many ailments, including upper respiratory tract infections, diarrhea, headache, fever, eye problems, skin diseases, and pneumonia.[13–15] The oil is also a potent antidiabetic agent.[16,17] Mbata and Saikia have reported the use of *O gratissimum* for flavoring foods and as an antimicrobial agent.[18]

Blood is a tissue that consists of fluid plasma in which are suspended a number of formed elements. The blood cells exist at fairly constant levels, suggesting the existence of feedback

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Access this article online	
Quick Response Code:	Website: www.ijabmr.org
	DOI: 10.4103/2229-516X.96807

regulatory mechanisms.[19,20] The effect of *O gratissimum* on the homeostasis of blood cells is not backed by scientific evidence. With this study we hope to add to the knowledge regarding the effect of *O gratissimum* on hematological parameters, especially the platelet indices such as mean platelet volume (MPV), platelet distribution width (PDW), and platelet–large cell ratio (P–LCR), which are major determinants of ischemic heart disease.

Thus, this study was planned to investigate the effect of oral administration of *O gratissimum* on hematological parameters.

Materials and Methods

Preparation of plant extract

Four kilograms of fresh leaves of African basil were purchased from a local market in Calabar South Local Government of Cross River State, Nigeria, during the rainy season and were identified as *Ocimum gratissimum* by a botanist in the Department of Biological Sciences, University of Calabar, Nigeria. The leaves were first washed free of sand and debris. Wash water was blotted off and the leaves were ground to a paste. A quantity of the ground sample (50 g) was weighed and Soxhlet extracted with 150 ml distilled water at 100°C for 9 hours. The extract was slowly evaporated to dryness in vacuum at 40°C using a rotary evaporator. A total yield of 33.2% of the ground sample was obtained. Weighed samples (20 g in 10 ml distilled water) of the extract were then used to prepare the stock solution (500 mg/ml) as has been previously described.[21]

Experimental animals and feeding protocol

Thirty male albino Wistar rats were obtained from the animal house of the Department of Medical Physiology, University of Calabar, Nigeria. The research was conducted in accordance with the internationally accepted principles for laboratory animal use and care as found in, for example, the European Community guidelines.[22]

The rats were divided into three groups of ten rats each. They were fed as follows: group 1 (control) was fed only normal rat pellets and drinking water; group 2 (LD) was fed on normal rat pellets plus 500 mg/kg of extract orally once daily; group 3 (HD) was given normal rat pellets plus 1000 mg/kg of extract orally once daily. All rats were allowed free access to drinking water. The feeding regimens lasted for 4 weeks. At the end of the feeding period the animals were sacrificed and a blood sample was collected for analysis.

Collection of blood samples

The animals were made unconscious with chloroform inhalation (cotton wool soaked in 3.5% chloroform) and blood was collected via cardiac puncture using a 5-ml syringe attached to a needle (21 SWG); the blood was collected into plain capped bottles containing ethylenediaminetetraacetate (EDTA) by a modified method of Ohwada.[23] The samples were immediately used for the estimation of the different variables.

Measurement of blood parameters

Blood samples were analyzed using an automated cell counter (Coulter Electronics, Luton, Bedfordshire, UK) with standard calibration, according to the manufacturer's instructions for analysis of human blood[24] and accurately programmed for the analysis of red blood cell (RBC) count, total white blood cell (WBC) count, hemoglobins (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobins concentration (MCHC), RBC distribution width (RDW), MPV, PDW, and P–LCR.

Statistical analysis

Data were presented as mean ± standard deviation (SD). Data were analyzed using one-way analysis of variance (ANOVA) followed by the *post hoc* test (least square deviation). P value of less than .05 was declared as statistically significant.

Results

Effect of *Ogratissimum* on RBC, PCV, and Hb in rats

The mean RBC count of the control, LD, and HD groups were 7.63 ± 0.78, 7.88 ± 0.66, and 9.07 ± 0.16 × 10^6/µl respectively. The HD group had a significantly (P<.01) higher RBC count compared with the control and LD groups.

The mean PCV was 48.88 ± 2.06% in the control group, 50.00 ± 4.62% in the LD group, and 55.26 ± 1.28% in the HD group. The PCV of the HD group was significantly higher than that of the control (P<.001) and LD (P<.01) groups.

The mean Hb concentrations were 14.66 ± 0.56 g/dl, 14.90 ± 0.24 g/dl, and 16.8 ± 0.72 g/dl for the control, LD, and HD groups, respectively. The HD group had a significantly (P<.001) higher Hb concentration than the control and LD groups.

Effect of *O gratissimum* on platelet count and total WBC count in rats

The total WBC counts were not significantly altered following extract administration [Figure 1].

The HD group had a mean platelet count of 928.40 ± 140.41 × 10^3 cells /µl, which was significantly (P<.05) higher than the values obtained for the control (715.00 ± 105.58 × 10^3 cells / µl) and LD (756.20 ± 64.15 × 10^3 cells /µl) groups [Figure 2].

Table 1: Effect of *O gratissimum* on red blood cell and platelet indices in rats

Parameter	Control	Low dose (LD)	High dose (HD)
MCV (fl)	64.46 ± 5.42	63.42 ± 1.48	61.04 ± 1.47[a]
MCH (pg)	19.34 ± 1.72	18.48 ± 0.71	18.66 ± 0.90
MCHC (g/dl)	29.98 ± 0.51	29.18 ± 1.34	30.44 ± 0.73
RDW–SD (fl)	35.60 ± 3.17	37.46 ± 5.82	32.00 ± 1.61*
RDW–CV (%)	14.62 ± 1.33	16.02 ± 3.06	14.44 ± 1.92
MPV (fl)	7.28 ± 0.25	7.34 ± 0.21	7.00 ± 0.24[a]
P–LCR (%)	7.88 ± 1.49	8.92 ± 1.29	6.74 ± 1.37[a]
PDW (fl)	8.64 ± 0.47	9.08 ± 0.40	8.44 ± 0.27[a]

*P<.05 vs control; a P<.05 vs LD, MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; RDW–SD: RBC distribution width–standard deviation and RDW–CV: RBC distribution width–coefficient of variation MPV: Mean platelet volume; P–LCR: Platelet–large cell ratio; PDW: Platelet distribution width

Effect of O gratissimum on RBC indices in rats

The MCV for the control group was 64.46 ± 5.42 fl, while the values for the HD and LD were 63.42 ± 1.48 fl and 61.04 ± 1.47 fl, respectively. The MCV of the HD group was significantly (P<.01) higher than that of the LD group. However, the MCV values of the HD and LD groups were not significantly different from that of the control group. The MCH and MCHC of the control, LD, and HD groups were not significantly different from one another. The RBC distribution width–standard deviation (RDW–SD) and RBC distribution width–coefficient of variation (RDW–CV) of the three groups were also not significantly different from one other [Table 1].

Effect of *O gratissimum* on platelet indices in rats

The mean MPV of the control, LD, and HD groups were 7.28 ± 0.25 fl, 7.34 ± 0.21 fl, and 7.00 ± 0.24 fl, respectively. The MPV of the HD group was significantly (P<.05) lower than that of the LD group. However, the MPV of the HD and LD groups were not significantly different from that of the control group. The control group had a mean P–LCR of 7.88 ± 1.49 fl, while the mean P–LCR was 8.92 ± 1.29 fl and 6.74 ± 1.37 fl in the HD and LD groups, respectively. The P–LCR of the HD group was significantly (P<.05) lower than the P–LCR of the LD group. However, the P–LCR of the HD and LD groups were not significantly different from the value obtained for the control group. The mean PDW values were 8.64 ± 0.47 fl, 9.08 ± 0.40 fl, and 8.44 ± 0.27 fl, respectively, for the control, LD, and HD groups. The PDW was significantly (P<.05) lower in the HD group compared with the LD group. However, there was no significant difference between HD and control, or LD and control [Table 1].

Effect of O gratissimum on differential WBC count in rats

No significant changes were observed in the proportion of monocytes, eosinophils, and basophils following oral administration of the extract. However, neutrophils were significantly (P<.05) elevated in the HD group (23.40 ± 1.14%) as compared with the LD group (21.40 ± 0.55%), while the lymphocyte count was significantly (P<.05) lower in the HD group (66.00 ± 0.58%) relative to the LD extract recipients (68.00 ± 1.00%). No significant differences were observed in neutrophils and lymphocytes counts between the LD and the control groups [Table 2].

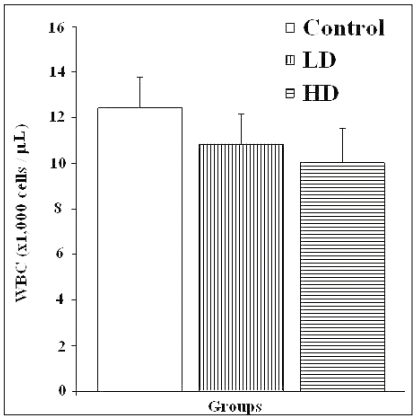

Figure 1: Comparison of total white blood cell (WBC) count in different experimental groups

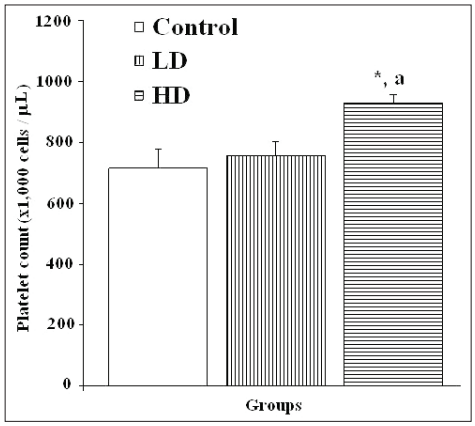

Figure 2: Comparison of platelet count in different experimental groups (*P<.05 *vs* control; [a]P<.05 *vs* LD)

Discussion

The effect of *O gratissimum* on hematological parameters in rats was investigated in this study. The extract (at high dose) caused

Table 2: Effect of *O gratissimum* on differential white blood cells in rats

Parameters	Control	Low dose	High dose
Neutrophils	22.60 ± 1.14	21.40 ± 0.55	23.40 ± 1.52[a]
Lymphocytes	66.60 ± 1.82	68.00 ± 1.00	66.00 ± 1.58[a]
Basophils	0.60 ± 0.55	0.20 ± 0.45	0.40 ± 0.55
Monocytes	6.40 ± 0.55	6.80 ± 0.84	6.60 ± 1.52
Eosinophils	3.80 ± 0.84	3.60 ± 0.55	3.60 ± 0.55

[a]*P*<.05 *vs* Lose dose

an increase in the erythrocyte count. This was confirmed by the increased hematocrit (PCV) and percentage Hb in the high-dose recipient group. In normal circumstances, local tissue anoxia apparently leads to the formation of a glycoprotein called erythropoietin, which stimulates increased production of erythrocytes.[20] It is very likely that *O. gratissimum leaves* extract contains erythropoietin-like agent(s) which is/are responsible for the increased production of erythrocytes.

Similar results were obtained for thrombocytes (platelets). There was thrombocytosis in the group that received high dose of the extract. Therefore, it would seem likely that the extract also contains some compounds that are capable of causing the release of a thrombopoietin,[25]. Platelets play an important role in the maintenance of normal homeostasis and MPV is an indicator of platelet function, including platelet aggregation; release of thromboxane A_2, platelet factor 4, and beta- thromboglobulin; and expression of glycogen 1b and glycogen IIb/IIIa receptors.[26–30] In this study, MPV decreased in recipients of high-dose *O gratissimum*. MPV, as a determinant of platelet function, is a newly emerging indicator of risk for atherothrombosis. Increase in MPV has been documented in patients with metabolic syndrome, stroke, and diabetes mellitus.[31,32] Many studies have shown that increased MPV is one of the risk factors for myocardial infarction, cerebral ischemia/transient ischemic attacks, and chronic vascular disease.[33–37]

We also observed in this study that RDW–SD was significantly reduced in the HD group as compared with the LD group. RDW–SD is a numerical measure of the variability in size (anisocytosis) of circulating erythrocytes.[38] This parameter is routinely reported as part of the complete blood count but its use is generally restricted to narrowing the differential diagnosis of anemia.[39] There is a strong correlation between RDW and the risk of adverse outcome of heart failure.[40] It is also elevated in thrombotic thrombocytopenic purpura, a disease of unknown origin, which is characterized by abnormally low levels of platelets in the blood, formation of blood clots in the arterioles and capillaries of many organs, and neurological damage.

The total WBC (leucocyte) counts were not significantly altered following extract administration. However, examination of the differential counts revealed that high doses of the extract led to reduction in lymphocyte count but increased the neutrophil count in the rats; thus, the total WBC count remained largely unaltered. The reduction in lymphocyte count could probably be due to cell margination rather than destruction. It is also possible that the extract contains agents that stimulate the bone marrow to produce neutrophils and release them into the blood. Neutrophils are the major granulocytes to be activated when the body is invaded by bacteria and they provide the first line of defense against invading microorganisms.[41] The granules of the neutrophil contains many enzymes, which makes it a powerful and effective killer machine and, hence, deficiency of neutrophils in the body leads to myriad defects, including conditions such as chronic granulomatous disease. This effect on neutrophil count may be partly responsible for the claim that *O gratissimum* has antibacterial actions.[11,12]

Our study is at variance with the earlier study by Jimoh *et al.* who reported decrease in these same hematological parameters following administration of *O gratissimum* in Wistar rats.[42] They attributed the decrease to the presence of saponins in the extract.

In another study with results contradictory to ours, Obianime *et al.* reported that aqueous *O gratissimum* leaf extract (11–88 mg/kg) decreased PCV and Hb levels in the first week, whereas WBC and lymphocyte counts were increased during the first and second weeks, respectively, in male mice. They attributed the reduced hematological effects of *O gratissimum* to stimulation of adaptive mechanisms within the body against *O gratissimum*–induced toxicity on the blood cells. They suggested that *O gratissimum* may have two different effects—oxidative or antioxidative—depending on the tissue/organ system under investigation and the duration of administration.[43]

To summarize, from this study we conclude that oral administration of *O gratissimum* increases red blood cell count, packed cell volume, hemoglobin level, platelet count, and the proportion of neutrophils, and also leads to decrease in platelet indices (i.e., MPV, P–LCR, and PDW) at high doses.

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How to cite this article: Ofem OE, Ani EJ, Eno AE. Effect of aqueous leaves extract of Ocimum gratissimum on hematological parameters in rats. Int J App Basic Med Res 2012;2:38-42.

Source of Support: Nil. **Conflict of Interest:** None declared.