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# Consumption of Aqueous Leaf Extract of *Ocimum gratissimum* Impairs Haematological Function in Wistar Rats

**Aribo EO*, Udefa AL**
Department of Physiology, College of Medical Sciences, University of Calabar, Calabar, Nigeria

***Corresponding author**
*Aribo EO*

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**Abstract:** There have been conflicting reports by researchers on the effect of aqueous leaf extract of *Ocimum gratissimum* (OG) on haematological parameters This study was carried out to evaluate the dose-dependent effect of OG leaf extract on some haematological parameters in wistar rats. Twenty-four male rats were randomly assigned into four groups of six rats each. Group 1 was a control while groups 2, 3 and 4 were test groups ($T_1 – T_3$) and were given 450mg/kg, 800mg/kg and 1800mg/kg respectively of OG leaf extract daily for 30 days. All rats had free access to water and rat chow. Results showed a significantly decreased RBC count in $T_2$ ($p<0.05$) and $T_3$ ($p<0.01$) compared with control. Platelet count and PCV were significantly decreased in $T_3$ ($p<0.05$) compared with control. A significant increase in total WBC count was observed in $T_1$ ($p<0.05$) and $T_2$ ($p<0.01$) when compared with control. A significant reduction in neutrophil count in $T_1$ ($p<0.05$), $T_2$ ($p< 0.05$) and $T_3$ ($p<0.01$) compared with control was observed. Lymphocyte count was significantly increased in $T_1$ ($p<0.05$), $T_2$ ($p<0.01$) and $T_3$ ($p<0.01$) compared with control. There was no significant difference in MCV, MCH and MCHC in the different groups. In conclusion, aqueous leaf extract of *Ocimum gratissimum* caused reduction in RBC count, PCV, platelet count and neutrophil count but caused increases in total WBC count and lymphocyte count.
**Keywords:** *Ocimum gratissmum*, haematological parameters, aqueous leaf extract.

## INTRODUCTION

*Ocimum gratissimum* or "scent leaf" is an aromatic perinneal shrub. It is called "nchawu" in Igbo, "nton" and "efinrin" by Efiks and Yorubas respectively. It is found throughout the tropics and sub-tropic regions where it is widely consumed because of its nutritional and medicinal values [1]. It is used as a seasoning due to its aromatic flavour. It is also known to have several medical effects including hypoglycaemia on alloxan-induced diabetic rats [2] and antimicrobial activity [3]. Other medicinal effects include antidiarrhoeal [4], anticancer [5], antispasmodic [6], analgesic [7], aphrodisiac [8] and anti-helminthic [9] actions. Its ethanolic extract is hepatoprotective [10] and also possesses antioxidant [11] and antipyretic [12] activities.

The extract of Ocimum gratissimum or its oil contains eugenol [11], non-cyclic sesquiterpenes [13], tannins, phytates, alkaloids, flavonoids [14] as well as phenols associated with antioxidant activity [15].

Though some work has been done on the nutritional and medicinal effects of the leaves of this plant, there are conflicting results on haematological changes that occur following its long term consumption. It therefore becomes necessary to investigate this aspect of the effects of *Ocimum gratissimum* as a way of contributing to the growing knowledge of this plant.

## MATERIALS AND METHODS

### Plant material and preparation of extract

The fresh leaves of *Ocimum gratissimum* (OG) purchased from Watt Market, Calabar, Nigeria were identified by the Chief herbarium of the University of Calabar. The leaves were washed free of sand, debris and water, blotted off and then dried in an oven (Astell Hearson) at 40-45°C. It was blended and 2000g of the powder soaked in 3.5 litres of water for 12 hours the mixture being stirred at interval and then filtered with Whatman filter paper size 1. The filtrate was evaporated in an oven (Astell Hearson) at 40-45°C to obtain an extract. Stocks were prepared by dissolving 3g, 4g and 6g of the extract in 10ml of water to produce concentrations of 300mg/ml, 400mg/ml and 600mg/ml respectively.

### Experimental animals

Twenty-four Wistar rats were used for the study. The rats were bought from the Department of Agriculture, University of Calabar and housed in the animal house of Department of Physiology, University of Calabar. They were fed with rat chow and had free access to water throughout the thirty-day duration of administration. The Ethics Committee of the Faculty of

Basic Medical Sciences, University of Calabar approved the study.

**Experimental design**

The rats were randomly assigned into four groups of six rats each with group 1 as control and groups 2 to 4 as test groups [Test 1 ($T_1$), Test 2 ($T_2$) and Test 3 ($T_3$). The test groups, $T_1$, $T_2$ and $T_3$ received 450mg/kg, 800mg/kg and 1800mg/kg respectively of OG leaf extract which was given as a single daily dose by gavaging for thirty days. The cages were hygienically maintained.

**Collection of blood samples**

The rats were anaesthetized with 3.5% chloroform at the expiration of duration of administration and blood samples collected by intra-cardiac puncture into labelled EDTA bottles for haematological analysis.

**Haematological analysis**

Haematological parameters were determined using the method by Baker and Silverton [16]. Total RBC count was done with haemocytometer using a solution of 3.13% trisodium citrate as diluents, count determined with a formula which integrated the number of RBC counted, dilution factor as well as area and depth of chamber. The total WBC count was performed with same method [16] and the horizontal method as used the medthod by Baker and Silverton [16] was used for the differential WBC count. Platelets count was also done according to the method of Baker and Silverton [16] using haemocytometer after 1 in 20 dilution of sample in 1% ammonium oxalate.

The PCV was determined using the hematocrit technique and read with a hematocrit reader [17]. The mean corpuscular hemoglobin concentration (MCHC) was assessed by computation using the values of PCV and Hb concentration [17] while the mean corpuscular hemoglobin (MCH) was determined by computation as function of hemoglobin concentration and RBC count [17]. The mean corpuscular volume (MCV) was derived by computation as a function of PCV and RBC count [17].

**Statistical analysis**

Results are expressed as mean ± standard error of mean (SEM) and were analysed using Statistical Package for Social Science (SPSS) (version 21). Comparison of control and test values was performed using one-way analysis of variance (ANOVA) and followed with a post hoc test of multiple comparison. $p<0.05$ was considered statistically significant.

**RESULTS AND DISCUSSION**

Figures-1, 2 and 3 show RBC count, PCV and platelet count in the different groups. There was a significant decrease in RBC count in $T_2$ ($p<0.05$) and $T_3$($p<0.01$) compared with control (Fig-1). The PCV in $T_3$ was significantly ($p<0.05$) decreased compared with control (Fig-2). Platelet count was significantly ($p<0.05$) decreased in $T_3$ compared with control (Fig-3).

Figures-4 and 5 show total white blood cell (WBC) and differential white blood cell count in the different groups. Total WBC count was significantly increased in $T_1$ ($p<0.05$) and $T_2$ ($p<0.01$) compared with control (Fig-4). Neutrophil count was significantly decreased in $T_1$ ($p<0.05$), $T_2$ ($p<0.05$) and $T_3$ ($p<0.01$) compared with control (Fig-5). Lymphocyte count was significantly increased in $T_1$ ($p<0.05$), $T_2$ ($p<0.01$) and $T_3$ ($p<0.01$) compared with control (Fig-5).

Table-1 shows absolute red blood cell values in the different groups. There was no significant difference in MCV, MCH and MCHC among the different groups.

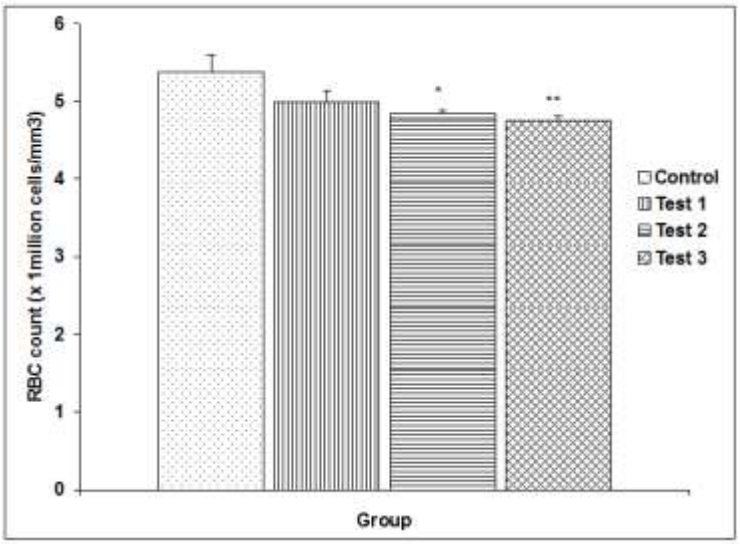


**Fig-1: Comparison of the red blood cell count in control and test groups. Values are mean ± SEM, n=5. *p<0.05, **p<0.01, vs control.**

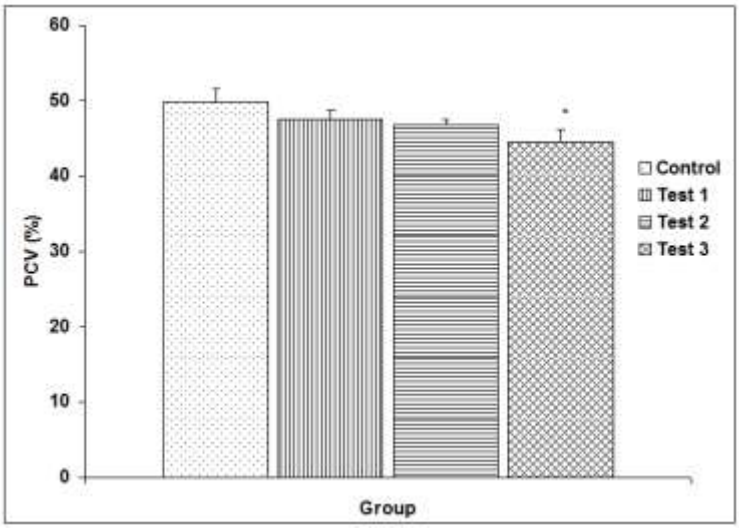


**Fig-2: Comparison of the cell volume in control and tests groups. Values are mean ± SEM, n=5. *p<0.05 vs control.**

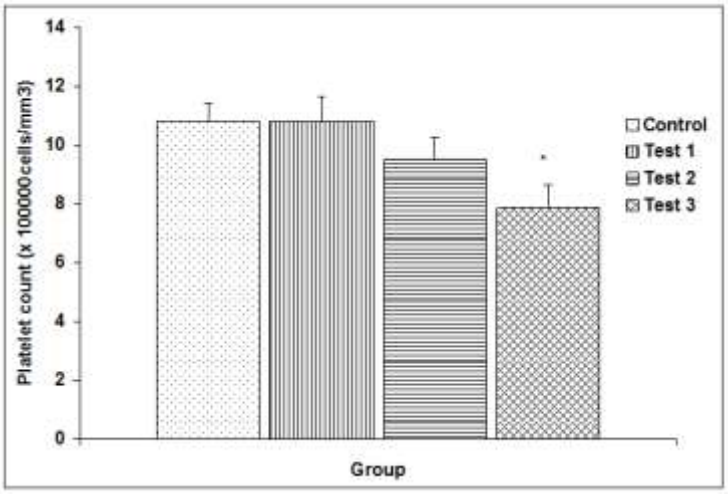


**Fig-3: Comparison of the platelet count in control and tests groups. Values are mean ± SEM, n=5. *p<0.05 vs control.**

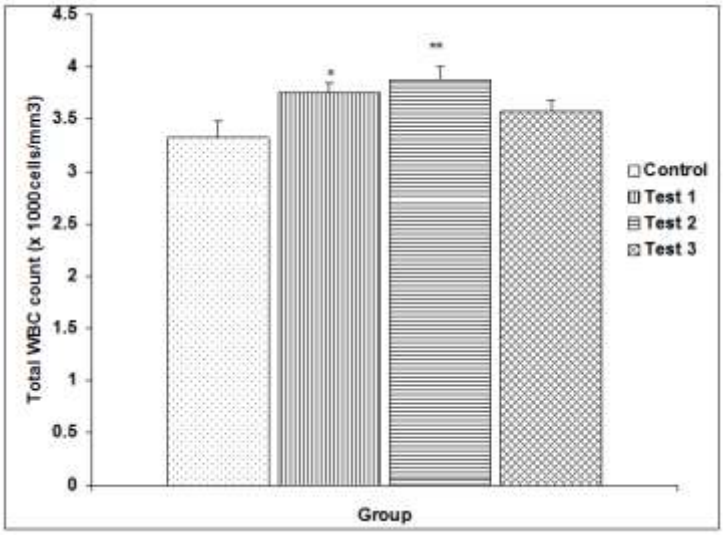


**Fig-4: Comparison of the total white blood cell count in control and tests groups. Values are mean ± SEM, n=5. *p<0.05, **p<0.01 vs control.**

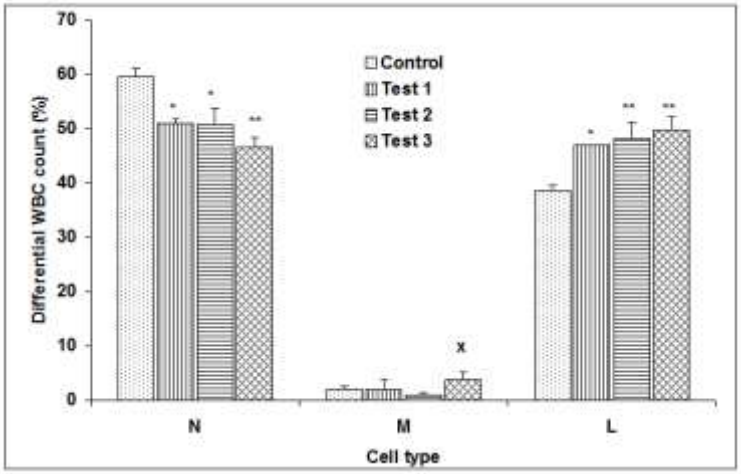


**Fig-5: Comparison of the differential white blood cell count in control and tests groups. Values are mean ± SEM, n=5. *p<0.05, **p<0.01 vs control.**

**Table-1: Comparison of absolute red blood cells values in the different experiment groups**

| | MCV | MCH | MCHC |
|---|---|---|---|
| **Control** | 92.63 | 30.73 | 33.38 |
| | ±3.14 | ±1.07 | ±0.77 |
| **Test 1** | 95.10 | 31.93 | 33.53 |
| | ±1.48 | ±0.50 | ±0.11 |
| **Test 2** | 93.45 | 31.35 | 33.13 |
| | ±0.12 | ±0.36 | ±0.13 |
| **Test 3** | 95.50 | 31.95 | 33.50 |
| | ±2.99 | ±1.17 | ±0.19 |

Values are expressed as mean ±SEM, n = 5.
No significant differences among groups

The study demonstrated that aqueous leaf extract of *Ocimum gratissimum* caused a reduction in RBC count in rats fed with the extract and also a decrease in PCV and platelet count in the group that received the highest dose (i.e. $T_3$). Whereas there was a reduction in neutrophil count in all extract treated groups, an increase in lymphocytes count was observed in all extract treated groups. These results are similar to those of Jimoh *et al.*[18] who observed significant decreases in Hb, RBC count and PCV in extract treated rats and also a decreased MCH and WBC count. Obaji *et al.*[19] also observed significant reductions in RBC count, Hb concentration and PCV in a dose-related manner. Obianime *et al.* [20] in their chronic toxicity study observed significant reductions in Hb and PCV after 2 weeks of extract treatment but with no significant difference in haematological parameters in all groups after 4 weeks of extract feeding. Our findings are not in consonance with those of Ofem *et al.*[21] who rather observed increases in PCV, Hb, and RBC count at high dose. Our results showed an increase in WBC count in high dose groups similar to Obianime *et al.*[20] finding after 2 weeks of feeding but different from Obaji *et al.*[19] and Jimoh *et al.*[18] who noted an increase in WBC and Ofem *et al.*[21] who did not observe any difference in WBC count. Our observed decrease in platelet count in HD is at variance with Ofem *et al.*[21] who noticed an increase in platelet count and Jimoh *et al.*[18], Obaji *et al.*[19] and Obianime *et al.*[20] who did not observe any difference in platelet count. We observed no significant effect of the extract at high doses on MCV, MCH and MCHC similar to the findings of Ofem *et al.*[21], Jimoh *et al.*[18] and Obianime *et al.*[20].

The variations in some of these findings may have to do with the possible use of different subtypes or species of *Ocimum gratissimum*, use of plants grown on different soils which affect their phytochemistry [22, 23] as well as duration of administration of extract. It might have been also due to the wide variation in dosages used by experimenters (inter-researcher).

The aqueous leaf extract of *Ocimum gratissimum* contains alkaloids, tannins, phenols, flavanoids, saponins [13] as well as cyanogenic compounds [14]. The observed decline in haematological parameters could point to some degree of toxicity to the hematopoietic system possibly by cyanogenic compounds which are known to have adverse effects on hematopoietic system [24]. The increase in lymphocyte count could be the result of selective and adaptive response of the hematopoietic tissues to chronic ingestion of the extract.

## CONCLUSION

This study therefore has further thrown more light on the possible haematotoxicity of *Ocimum gratissimum* which can lead to anaemia, platelet depletion and decline in neutrophil count with attendant clotting and immune challenges. It should therefore be consumed with caution.

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