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*Full Length Research Paper*

# Reduction of platelet and lymphocyte counts and elevation of neutrophil counts in rats treated with aqueous leaf extract of *Ocimum gratissimum*

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***Ocimum gratissimum* (Linn.) has been widely used for food and medicinal purposes. The effect of aqueous leaf extract of *O. gratissimum* on haematological parameters of albino Wistar rats was studied. Acute toxicity study showed a median lethal dose ($LD_{50}$) value of 2121.32 mg/kg body weight for intraperitoneal (i. p) route in mice. Twenty rats' weights ranging between 85 to 115 g were used. The rats were divided into four groups; with group 1 as the control group. Increasing doses (212, 424 and 636mg/kg body weight) of extract were administered orally to the three groups for a period of 21 days. Significant ($p<0.001$ and $p<0.01$) increases of WBC in groups II and III and significant ($P<0.05$) decreases in platelet count and lymphocyte levels were observed. Similarly, a significant ($P<0.05$) increase in the neutrophil levels was observed. There were no significant changes in red blood cell (RBC), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC). *O. gratissimum* reduces platelet and lymphocyte counts, while it increases total WBC and neutrophil levels. It is evident from this study that there is need for caution with the consumption or administration of excessive dosage of *O. gratissimum*.**

**Key words:** *Ocimum gratissimum*, toxicity, platelet, lymphocyte, neutrophil.

## INTRODUCTION

Man throughout the ages has depended on his immediate environment for food and medication. Most especially, man has consistently resorted to plants for solution to the myriad of health problems challenging him. Traditional herbal practitioners have made several claims on numerous herbal preparations with specific claim on the efficacy of *Ocimum grastissimum* in the treatment of several disease conditions including infections, oncogenic and neurological disorders.

There is widespread usage and consumption of *O. gratissimum* globally. It is used as spice and is known to have enormous nutritional values. The leaves of the plant which is highly appreciated for its pleasant aroma is used for seasoning food and as vegetable. It contains high moisture, low protein, ash, vitamins A, $B_2$, and D, calcium, phosphorus, selenium, iron, zinc and magnesium (Oboh et al., 2009).

Phytochemical screening reveals that the volatile aromatic oil from the leaves of *Ocimum gratissimum* consists of thymol, Eugenol, terpenes, xanthones and lactones (Oboh, 2008).

Other reports had showed that this plant contains; α–pinene, β –pinene. 1, 8 – cineole, β – caryophyllene, a murolene and sehirene (Sainsbury and Sofowora, 1971). Other constituents include germacrene, x-copaene, humutene, β -elemene, β – bourbonenem and serinerel (Pande and Pathak, 2009). Regional variation in the phytochemical constituent of *Ocimum gratissimum* has been reported (Oboh et al., 2009).

The widespread usage of this plant is informed by its peculiar pharmacological properties such as its ability to scavenge free radical as antioxidant (Odukoya et al., 2005; Akinmoladun et al., 2007; Aprioku and Obianime, 2008), antidiarrhoeal (Orafidiya et al., 2000; Adebolu and Salau, 2005), antihelmintic (Fakae et al., 2000; Pessoa et al., 2002). Its content of thymol makes it a more

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acceptable antiseptic agent (Agnaniet et al., 2005) particularly by the local folks.

Blood is a specialized connective tissue that contains cells suspended in a fluid extracellular matrix called plasma, making it the main target for any detrimental effect(s) of *O. gratissimum* extract. The RBC plays a primary role of transporting substances including nutrients, respiratory gases and other waste materials throughout the body. The WBC defends the body against pathogens and other foreign bodies. The platelets play the role of preventing blood loss. Therefore, severe alteration in the concentration of any of these haemopoietic components may be detrimental.

Day after day, the consumption of *O. gratissimum* increases globally in view of its many uses. However, the usage of any plant, either as food or medication should be weighed against its possible detrimental effect(s) on the physiochemical integrity of the body. The fact that traditional medicine practitioners prescribe and administer decoctions of the leaves to clients regardless of the possible adverse effect of administration of unverified dosages on the body system and also considering the strategic place of the haemopoietic system in animal physiology. This study was conducted to investigate the possible effects of *O. grastissimum* on some of the haematological parameters as well as its safety for usage as food or medication by man.

## MATERIALS AND METHODS

### Collection and identification of plant materials

Fresh leaves of *O. gratissimu*m were collected from Ukanafun Local Government Area in Akwa Ibom State, Nigeria. The leaves were later identified and authenticated by a senior herbarium officer in the Department of Pharmacognosy, Faculty of Pharmacy, University of Uyo, Uyo.

### Preparation of extract

Fresh leaves of *O. gratissimum* were air-dried and ground into coarse powder. 450 g of the powdered leaves was macerated in 2000 ml of distilled water. The mixture was allowed for 24 h before filtration through a fine sieve. The filtrate was evaporated to dryness at 45°C using rotations evaporator. The extract was kept in a refrigerator at -4°C until use.

### Acute toxicity study

The median lethal dose ($LD_{50}$) of the plant extract was determined by method of Lorke (1983). The rats were divided into 4 groups and treated with dosage of 250, 500, 750, 1000 mg/kg body weight in the 1st phase and dosage of 1500, 1750, 2000 and 2250 mg/kg body weight intraperitoneally in the 2nd phase. The median lethal dose ($LD_{50}$) was calculated using the second phase, according to the formula:

Lethal dose ($LD_{50}$); $LD_{50} = \sqrt{(D_0 \times D_{100})}$

Where $D_0$ = Dosage of 0% mortality. $D_{100}$ = Dosage of 100% mortality.

### Experimental design and treatment of animals

The rats were weighed and kept in wooden cages of 50 to 80 cm dimension. They acclimatized for 1 week before the commencement of the experiment. The animals were fed with rat chow and allowed free access to water. Both sexes of rats were randomly assigned into 4 groups with group 1 as the control while group 2 to 4 were administered with the extract dosage of 212, 424, 636 mg/kg, respectively. The experimental procedures involving the animals and their care were conducted in conformity with the approved guidelines by the local Research and ethical Committee.

### Sample collection

After daily extract administration for 21 days, the rats were suffocated in chloroform on the 22nd day. Incisions were made into the ribs with a sterile pair of scissors to expose the heart. A sterile syringe with needle was used for collection of blood directly from the heart of each of the rats, by cardiac puncture. The blood sample was transferred into properly-labeled sample bottles with anticoagulant.

### Blood analysis

The blood samples were analyzed using an automated machine. Each of the samples collected were ran sequentially using the standard procedure for blood analysis. KX-21 haematological analyzer made by Symex Kobe Japan was used. Data obtained were analyzed using ANOVA and student t-test. Level of significance was pre-determined as $P<0.05$.

## RESULTS AND DISCUSSION

### Acute Toxicity Study

While animals that received very high dosage died, other signs of toxicity were noticed 2 to 4 h after extract administration. There was decrease locomotion, wrighting, constipation and decreased in sensitivity to touch. After 15 h of extract administration, the median lethal dose was calculated to be 2121.3 mmg/kg body weight.

### Analysis of blood parameters

The results of the different dosages of aqueous extract of *O. gratissimum* on the WBC, platelets, lymphocyte and neutrophils are shown in Table 1. The extract-treated groups showed significant ($p<0.001$ and $p<0.01$) increases for test groups II and III, respectively. The results also showed significant decrease in total platelet count ($P<0.001$) for groups II and IV, $p<0.05$ for group III when compared with the control group as shown in Table 1. Also, in the experimental groups, the mean value for lymphocytes decreased significantly ($P<0.001$) when compared with animals in the control group. The animals

**Table 1.** Effect of aqueous leaf extract of *Ocimum gratissimum* on white blood cells, platelets, lymphocytes and neutrophils.

| Group/treatment | WBC (x10$^3$/µL) | Platelets (x10$^3$/µL) | Lymphocytes (%) | Neutrophils (%) |
|---|---|---|---|---|
| I (Control) | 16.90 ± 0.24 | 945.20 ± 1.96 | 81.12 ± 0.20 | 18.68 ± 0.22 |
| II (212 mg/Kg) | 19.34 ± 0.18*** | 744.20 ± 1.00*** | 77.04 ± 0.56*** | 22.96 ± 0.28*** |
| III (424 mg/Kg) | 18.64 ± 0.69** | 963.40 ± 1.93* | 69.66 ± 0.28*** | 20.34 ± 0.22* |
| IV (636 mg/Kg) | 16.90 ± 0.24 | 783.40 ± 1.03*** | 71.58 ± 0.49*** | 28.42 ± 0.25*** |

*** = Significantly different from group I (Control) at P < 0.001. ** = Significantly different from group I (Control) at P < 0.01. * = Significantly different from group I (Control) at P < 0.05.

**Table 2.** Effect of aqueous leaf extract of *Ocimum gratissimum* on *RBC*, PCV, Hb and haematological indices.

| Group/Treatment | RBC (x10$^3$/µL) | PCV (%) | Hb (g/dL) | MCV (fL) | MCH (pg) | MCHC (g/dL) | PCV (%) | Hb (g/dL) |
|---|---|---|---|---|---|---|---|---|
| I (Control) | 7.21 ± 0.28 | 44.16 ± 1.17 | 12.50 ± 0.36 | 61.18 ± 0.96 | 17.40 ± 0.41 | 28.02 ± 0.35 | 44.16 ± 1.17 | 12.50 ± 0.36 |
| II (212 mg/Kg) | 7.11 ± 0.22 | 46.68 ± 0.92 | 12.40 ± 0.32 | 61.84 ± 0.62 | 17.44 ± 0.15 | 28.06 ± 0.43 | 46.68 ± 0.92 | 12.40 ± 0.32 |
| III (424 mg/Kg) | 7.22 ± 0.18 | 45.44 ± 0.92 | 12.52 ± 0.35 | 61.74 ± 0.70 | 17.38 ± 0.39 | 27.48 ± 0.30 | 45.44 ± 0.92 | 12.52 ± 0.35 |
| IV (636 mg/Kg) | 7.55 ± 0.12 | 47.16 ± 1.61 | 13.06 ± 0.25 | 62.06 ± 1.28 | 17.06 ± 0.22 | 28.36 ± 0.34 | 47.16 ± 1.61 | 13.06 ± 0.25 |

All values are the mean ± SEM.

in the extract-treated groups showed a significant ($p<0.001$) increase in the mean value of neutrophils of groups II and IV when compared to the control group but was significantly ($p<0.05$) higher in group III.

The administration of *O. gratissimum* to rats did not show any significant change in RBC, PCV, Hb, MCV, MCH and MCHC, as shown in Table 2.

Administration of *O. gratissimum* (OG) to rats did not have any significant effect on RBC, PCV, Hb, MCV, MCH and MCHC as reported in this study. However, the WBC counts showed significant higher values in the tested groups II and III when compared with the control group; these data are in agreement with the data reported by Ephraim et al. (2000). These increases might be attributed to the homeostatic response by the endogenous defense system to the adverse effects of *O. gratissimum*.

The significant decrease in platelets count recorded in this study may be due to the toxic effect of the chemical properties of *O. gratissimum*. *O. gratissimum* is reported to reduce platelets count most probably because of its phytochemical saponins and cardiac glycosides content (Tohti et al., 2006).

The lymphocyte level at the end of the experiment was 81.12% on the average for the control group and 77.04, 69.66 and 71.58% on the average for the test groups. These significant reductions in the lymphocyte level of the tested groups as compared to the control group are in agreement with the findings of Ephraim et al. (2000) and Jimoh et al. (2008).

Contrary to the reports of Ephraim et al. (2000) and Jimoh et al. (2008), the neutrophil level significantly increased in the test groups when compared to the control group. On the basis of the present study, the increase neutrophil level re-emphasize its anti-bacterial and anti-fungal properties and justifies the use of the plant by traditional medicine practitioners; considering that neutrophils constitute the first line of defense. However, it is feared that proloned consumption of *O. gratissimum* could result in thrombocytopenia, leading to bleeding disorders, considering its ability to decrease platelet aggregation and platelet count. Meanwhile, increased neutrophils could offer some physiological advantage in neutrophil-mediated defense mechanisms. Further studies may be necessary to give more information about the effects on lymphocytes and neutrophils.

Obaji et al. (2009) had reported generalized anti-haematinic effects of *O. gratissimum* and cautioned on the adverse consequences of its prolonged usage. It is possible that *O. gratissimum* could induce haemolysis and can

even suppress haemopoiesis because of its phytochemical constituent of saponin, but the detail specific mechanism of action is not clear. Nevertheless, it appears that the anti-haematinic effect of saponins to some extent could be cell linage selective. There is also a possibility that other constituent of *O. gratissimum* could stimulate the activity of some haemopoietic growth factors while inhibiting others. It is known that some growth factors caused maturation of a single linage progenitor cells e.g. erythropoietin (EP) for erythrocytes, thrombopoietin (TPO) for thrombocytes and interleukin 9 (IL-9) for lymphoid cells (Reddy, 2008). It is therefore possible that some of the active agent in *O. gratissimum* could promote the action of growth factors related to neutrophil production, while at the same time inhibiting growth factors associated with platelets and lymphocytes production.

Contrasting results reported on similar studies in various regions tends to support the notion that, there may be regional differences in the phytochemical constituent of *O. gratissimum*. Again, there may be need to consider the dosage and duration of administration in each study, as this is likely to contribute to the conflicting results obtained from the different experimentation.

## Conclusion

It is evident that oral administration of aqueous leaf extract of OG reduces platelets and lymphocyte counts, but increases total WBC counts and neutrophil levels in wistar albino rats. If this result were to be extrapolated to human beings, then caution would be needed not to consume quantities of *O. gratissimum*. However, pharmacological studies are required to confirm the effect of *O. gratissimum* extract, without serve side effects.

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