



# **Prolonged Administration of Leaf Extract of *Ocimum gratissimum* Reduces RBC, PCV and Platelet Count but Increases Total WBC and Lymphocyte Counts in Rats**

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## **Authors' contributions**

*This work was carried out in collaboration among all authors. Authors EOA and MEM designed the study, wrote the protocol and wrote the first draft of the manuscript. Author OEO performed the statistical analysis, managed the analyses of the study and edited the manuscript. All authors read and approved the final manuscript.*

## **Article Information**

DOI: 10.9734/EJMP/2019/v27i230111

### Editor(s):

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Complete Peer review History: <http://www.sdiarticle3.com/review-history/14729>

**Original Research Article**

**Received 09 September 2014**

**Accepted 22 December 2014**

**Published 05 April 2019**

## **ABSTRACT**

**Background:** *Ocimum gratissimum* is an ethnopharmacological plant with numerous healing potentials. But there is paucity in scientific literature on its impact on the blood cells.

**Aim and Objective:** The present study was intended to evaluate the dose and time-dependent effect of aqueous leaf extract of *Ocimum gratissimum* on some haematological in albino Wistar rats.

**Methods:** Twenty four rats were randomly assigned to four equal groups. Group 1 was control, groups 2, 3 and 4 were given 450 mg/kg, 800 mg/kg and 1800 mg/kg respectively of the extract daily for 30 days. All rats had free access to water and rat chow.

**Results:** The result showed a significantly lower RBC count in groups 3 and 4 when compared with control ( $p < 0.05$  and  $p < 0.01$  respectively). PCV was significantly lower in group 4 than control ( $p < 0.05$ ). Platelet count was significantly lower in group 4 than control ( $p < 0.05$ ) and group 2

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( $p < 0.05$ ). A significant increase in WBC count was observed in groups 2 and 3 when compared with control ( $p < 0.05$  and  $p < 0.01$  respectively). A significant reduction in neutrophil count was observed in groups 2, 3 and 4 compared with control ( $p < 0.05$ ;  $p < 0.05$  and  $p < 0.01$  respectively). There were significant increases in lymphocyte counts in groups 2, 3 and 4 when compared with control ( $p < 0.05$ ,  $p < 0.01$  and  $p < 0.01$  respectively). There were no significant changes in MCV, MCH and MCHC in the groups.

**Conclusion:** In conclusion, aqueous leaf extract of *Ocimum gratissimum* causes reduction in RBC count, PCV, platelet count and neutrophil count but causes increases in total WBC count and lymphocyte count.

**Keywords:** *Ocimum gratissimum*; haematological parameters; aqueous leaf extract.

## 1. INTRODUCTION

*Ocimum gratissimum* or "scent leaf" is an aromatic perennial shrub. It is called "nchawu" in Igbo, "nton" and "efirin" by Efiks and Yoruba 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 [2,3] and antimicrobial activity [4]. Other medicinal effects include antidiarrhoeal [5,6], anticancer [7], antispasmodic [8], analgesic [9], aphrodisiac [10] and anti-helminthic [11] actions. Its ethanolic extract is hepatoprotective [12] and also possesses antioxidant [13] and antipyretic [14] activities.

The extract of *Ocimum gratissimum* or its oil contains eugenol [13], non-cyclic sesquiterpenes, [15], tannins, phytates, alkaloids, flavanoids [16] as well as phenols associated with antioxidant activity [17]. It is obvious that substances taken into the body may affect the blood in different ways.

Blood is a tissue fluid composed of plasma and formed elements (RBC, WBC and platelets). The red blood cells are known for the transport of gasses to and from the tissues, the white blood cells provide defence to the body and partake in immunity of the body to infections, while platelets are involved in blood clotting [18,19]. The quantity and quality of the blood constituents are maintained at relatively constant levels by some homeostatic processes, alteration in this mechanism (homeostatic imbalance) affects the body negatively.

Though so much work has been done on its nutritional and medicinal effects of *Ocimum gratissimum*, there is rather paucity of

information on its effects on haematological parameters following its prolonged consumption and a few available provide conflicting results. The aim of this study is therefore to investigate the effects of *Ocimum gratissimum* after prolonged administration on haematological indices in rats.

## 2. MATERIALS AND METHODS

### 2.1 Plant Material and Preparation of Extract

The fresh leaves of *Ocimum gratissimum* purchased from Watt Market, Calabar were identified by the Chief herbarium of the University of Calabar. The leaves were washed free of sand and debris and water blotted off after which were dried in an oven (AstellHearson) at a temperature of 40-45°C. The dried leaves were blended with Binatone blender and 2000 g of the powder soaked in 3.5 litres of water for 12 hours. This mixture was stirred at interval and then filtered with Whatman filter paper size 1. The filtrate was evaporated in an oven (AstellHearson) at 40-45°C to obtain an extract. Stocks were prepared by dissolving 3 g, 4 g and 6 g of the extract in 10 ml of water to produce concentrations of 300 mg/ml, 400 mg/ml and 600 mg/ml respectively [20].

### 2.2 Animals

Twenty four albino Wistar rats of both sexes were used for the study. They were housed in the animal house of Physiology Department, University of Calabar, fed with rat chow and had free access to water throughout the thirty day duration of administration.

### 2.3 Experimental Design

Twenty four (24) male albino Wistar rats initially weighing between 120 g to 160 g were randomly

assigned into four groups of six rats. Group 1 was control, fed on normal rat chow and drinking water. Groups 2, 3 and 4 in addition to the control diet received 450 mg/kg, 800 mg/kg and 1800 mg/kg respectively of the extract orally and once daily. The feeding regimens lasted for thirty days. All the animals had free access to normal rat feed and clean drinking water. The cages were hygienically maintained. The rats were anaesthetized with chloroform at the expiration of duration of administration and blood samples collected by intra-cardiac puncture put into labelled EDTA bottles for haematological analysis.

## 2.4 Haematological Assay

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. Platelets count was done according using haemocytometer after 1 in 20 dilution of sample in 1% ammonium oxalate. The total WBC count was performed with same method while the horizontal method was used for the differential WBC count. Hemoglobin concentration was also determined using 1 in 20 dilution and at a wavelength of 540 nm, [21].

The PCV was determined using the hematocrit technique and read with a hematocrit reader. The mean corpuscular hemoglobin concentration (MCHC) was assessed by computation using the values of PCV and Hb concentration while the mean corpuscular hemoglobin (MCH) was determined by computation as function of hemoglobin concentration and RBC count. The mean corpuscular volume (MCV) was derived by computation as a function of PCV and RBC count [22].

## 2.5 Statistical Analysis

Data were expressed as mean  $\pm$  SEM. Comparison of control and test values was performed using one way analysis of variance (ANOVA). p-value less than ( $<0.05$ ) was considered statistically significant.

## 3. RESULTS

The red blood cell count for groups 1, 2, 3 and 4 were  $5.38 \pm 0.22$ ,  $5.00 \pm 0.14$ ,  $4.84 \pm 0.05$  and  $4.76 \pm 0.05 \times 10^6$  cell/mm<sup>3</sup>. There was a significant decrease in RBC count in groups 3 and 4

compared with control ( $p < 0.05$  and  $0.05$  respectively) as shown in Fig. 1.

The PCV (%) for groups 1, 2, 3 and 4 were  $49.75 \pm 1.89$ ,  $47.50 \pm 1.26$ ,  $46.75 \pm 0.75$  and  $44.50 \pm 1.71\%$  respectively. The PCV in group 4 was significantly lower compared with control  $p < 0.05$  (Fig. 2).

The platelet count was significantly ( $p < 0.05$ ) reduced in group 4 ( $7.86 \pm 0.77 \times 10^3$  cells/mm<sup>3</sup>) compared with control ( $10.80 \pm 0.61 \times 10^3$  cells/mm<sup>3</sup>) and group 2 ( $10.80 \pm 0.83 \times 10^3$  cells/mm<sup>3</sup>) as shown in Fig. 3.

A significant increase in total WBC count was observed in groups 2 and 3 when compared with control ( $p < 0.05$  in each) as shown in Fig. 4.

The mean neutrophil count was significantly lower in groups 2, 3 and 4 when compared with control at  $p < 0.05$ ,  $p < 0.05$  and  $p < 0.01$  respectively (Fig. 5). Lymphocyte counts were significantly increased in groups 2, and 4 when compared with control ( $p < 0.05$ ;  $p < 0.01$  and  $p < 0.01$  respectively) (Fig. 5).

There were no significant changes in MCV, MCH and MCHC when the different groups were compared with each other, (Tables 1).

**Table 1. Effect of *Ocimum gratissimum* leave extract on absolute red blood cells values in rats**

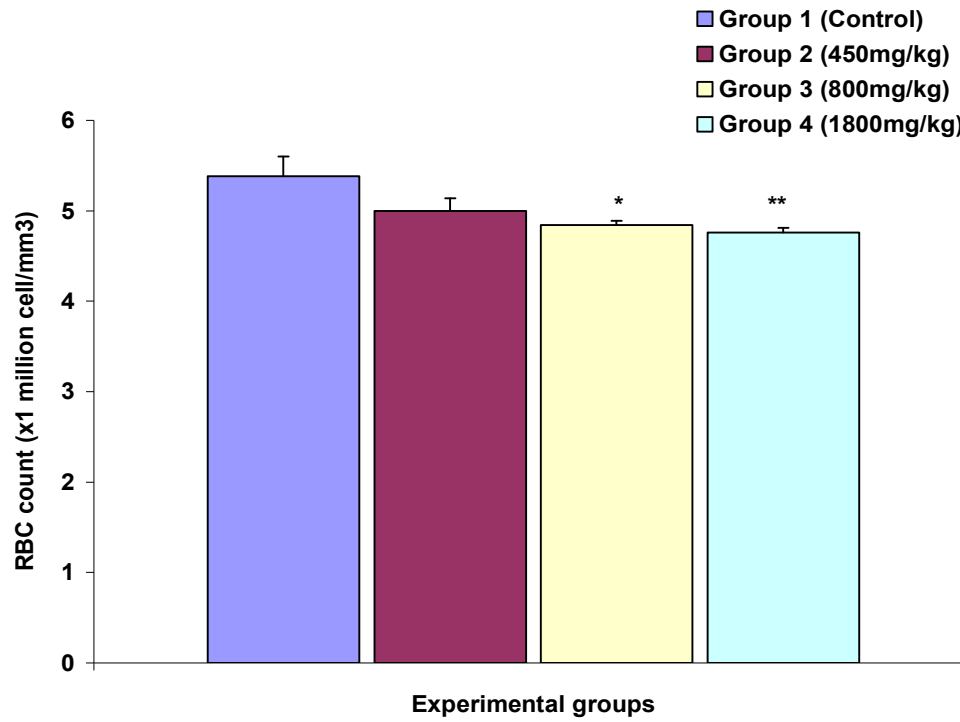
	MCV	MCH	MCHC
Group 1 (Control)	92.63 $\pm 3.14$	30.73 $\pm 1.07$	33.38 $\pm 0.77$
Group 2	95.10 $\pm 1.48$	31.93 $\pm 0.50$	33.53 $\pm 0.11$
Group 3	93.45 $\pm 0.12$	31.35 $\pm 0.36$	33.13 $\pm 0.13$
Group 4	95.50 $\pm 2.99$	31.95 $\pm 1.17$	33.50 $\pm 0.19$

Values are expressed as mean  $\pm$  SEM,  $n = 5$ .

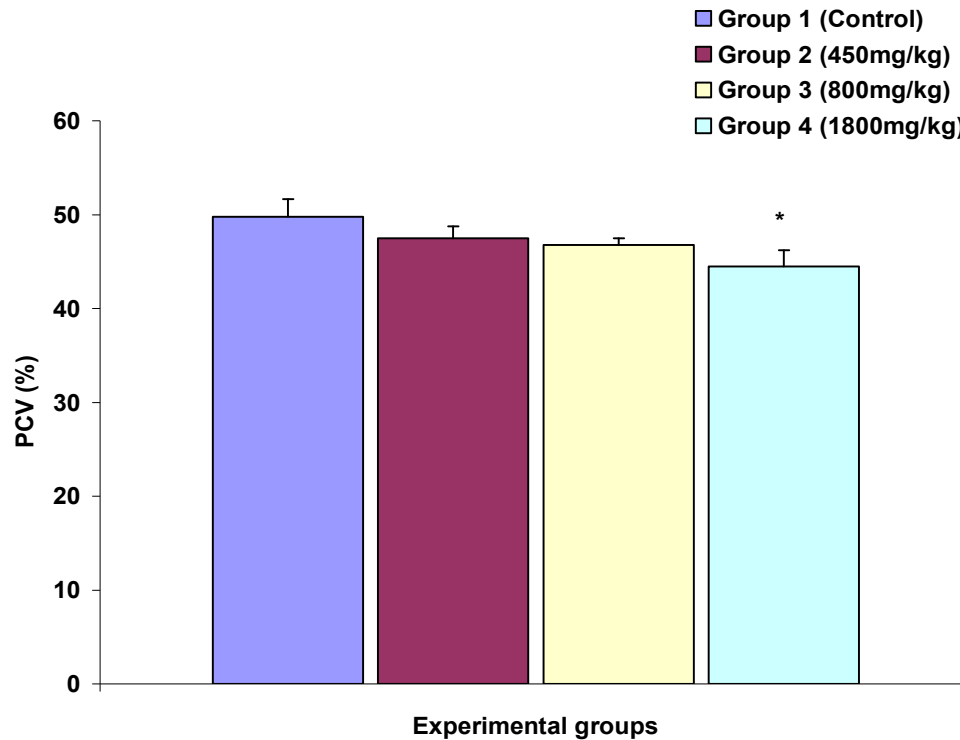
No significant differences among groups

## 4. DISCUSSION

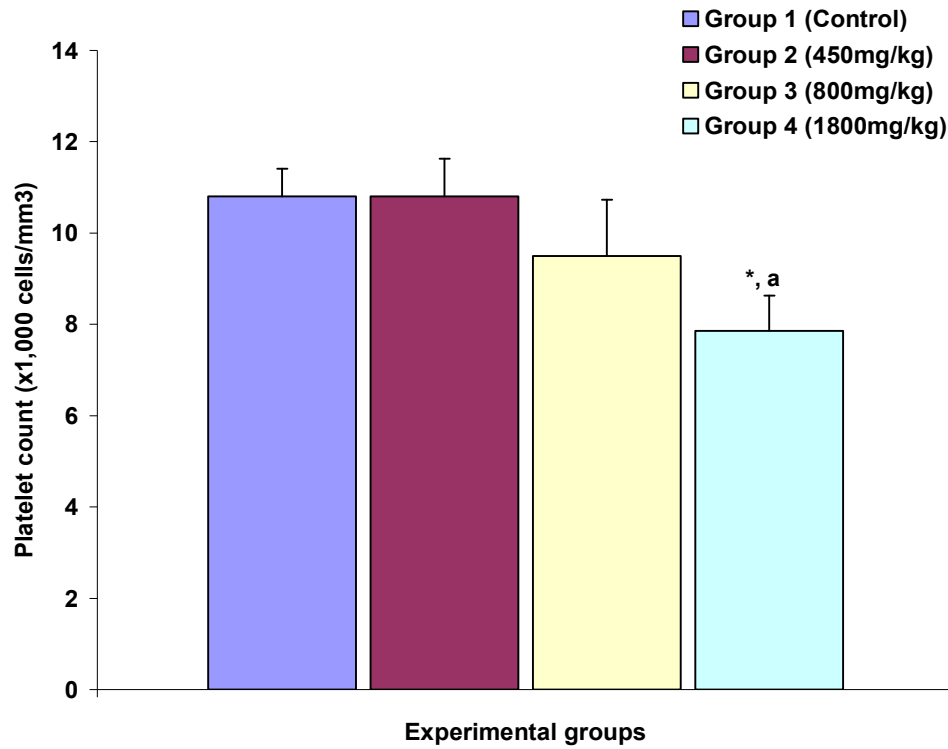
The study demonstrated that aqueous leaf extract of *Ocimum gratissimum* caused a reduction in RBC count in rats fed with higher doses of the tract and also a decrease in PCV and platelet count in the groups that received the highest doses i.e. T<sub>3</sub>. Whereas there was a reduction in neutrophil count in all extract treated groups, a decrease in lymphocytes count was observed in all extract treated groups.



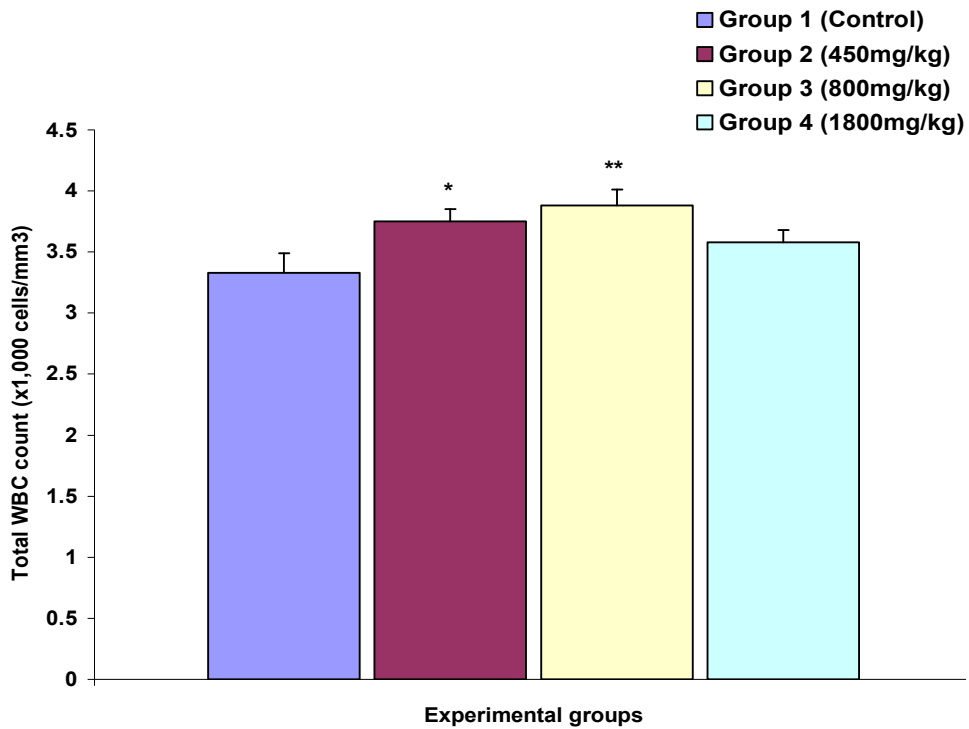
**Fig. 1. Effect of leaf extract of *Ocimum gratissimum* on red blood cell count in rats**  
 Values are expressed as mean  $\pm$  SEM, n=6. \*p<0.05, \*\*p<0.01 vs control



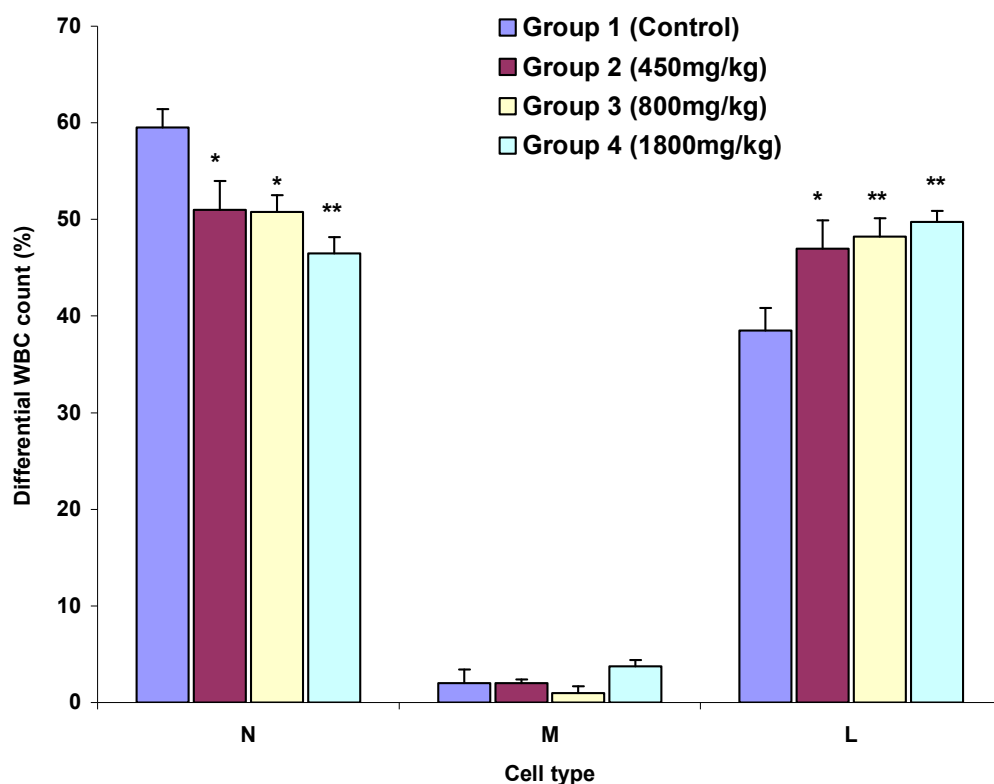
**Fig. 2. Effect of leaf extract of *Ocimum gratissimum* on packed cell volume in rats**  
 Values are expressed as mean  $\pm$  SEM, n=6. \*p<0.05vscontrol



**Fig. 3. Effect of leaf extract of *Ocimum gratissimum* on platelet count in rats**  
 Values are expressed as mean  $\pm$  SEM, n = 6. \*p<0.05 vs control; a = p<0.05 vs group 2



**Fig. 4. Effect of leaf extract of *Ocimum gratissimum* on total white blood cell count in rats**  
 Values are expressed as mean  $\pm$  SEM, n = 6. \*p<0.05, \*\*<0.01 vs control



**Fig. 5. Effect of leaf extract of *Ocimum gratissimum* on differential white blood cell in rat**

Values are expressed as mean  $\pm$  SEM,  $n = 6$ . \* $p < 0.05$ , \*\* $p < 0.01$  vs control

These results are similar to those of Jimoh and his colleagues [23] except for the increase in lymphocyte count but run contrary to those of Obinnime and his co-workers [24] in their chronic toxicity study. Our findings are also not in consonance with those of some investigators [20,25], who rather observed that *Ocimum gratissimum* increases PCV, Hb, RBC, neutrophil and platelet counts in rats but reduces the lymphocyte count compared with the control group.

The variations in some of these findings may have to do with the possible use of different subtypes or species of *Ocimum gratissimum* or it is possible that the *Ocimum gratissimum* used in this work was grown on different soils which could have affected their phytochemistry [26,27].

The aqueous leaf extract of *Ocimum gratissimum* contains alkaloids, tannins, phenols, flavanoids, saponins [15] as well as cyanogenic compounds [16]. Obviously, the levels of these compounds may vary depending on the location of the plant, which may in turn affect the efficacy of the extract. The observed reduction in RBC and PCV

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 [28,29].

Lymphocytes have been reported as the prevailing cells off the white blood cells, hence the increase in the total white blood cells observed in this study could possibly be due to increase in lymphocyte count. Lymphocytes play vital role in immune response of the body. They provide cell mediated and humoral immunity to the body.

The increase in lymphocyte count could be the result of selective and adaptive response of the hematopoietic tissues to chronic ingestion of the extract.

## 5. CONCLUSION

In conclusion, prolonged consumption of *Ocimum gratissimum* leaf extract leads to reductions in RBC count, PCV, platelet count and neutrophil count but causes increases in total WBC count and lymphocyte count. The extract

could therefore have the propensity to boost the immune system of the body.

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

## CONSENT

It is not applicable, because this study was conducted on experimental animals (albino Wistar rats).

## ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed. All experiments have been examined and approved by the appropriate University of Calabar Ethics Committee.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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