



## Ethanollic Extract of *Ocimum Gratissimum* (Scent Leaf) Leaves Improves Haematological Parameters in Restrain-Stressed Rats

<sup>1\*</sup>ANACHUNA, KK; <sup>2</sup>MOKE, EG; <sup>3</sup>OYEM, JC; <sup>2</sup>OMOGBIYA, AI; DAUBRY, <sup>1</sup>TME; <sup>1</sup>EBUWA, EI

<sup>1</sup>Department of Human Physiology, Faculty of Basic Medical Sciences, Delta State University Abraka, Delta State, Nigeria.

<sup>2</sup>Department of Pharmacology and Therapeutics, Faculty of Basic Medical Sciences, Delta State University Abraka, Delta State, Nigeria.

<sup>3</sup>Department of Human Anatomy and Cell Biology, Faculty of Basic Medical Sciences, University of Port Harcourt, Port Harcourt, Nigeria.

\*Corresponding Author Email: [goldasaba20@gmail.com](mailto:goldasaba20@gmail.com), Tel: +2348060410865

**ABSTRACT:** Stress alters system physiology as well as humoral immunity and hematology, and its problems have become a global issue. The present study was designed to investigate effects of the use of traditional herbs such as *Ocimum gratissimum* (OG) on hematological parameters in stressed rats. We subjected adult female Wistar rats to prolonged restrained stress for 14 days and administered 100 mg/kg, 200 mg/kg, 400 mg/kg of OG leaf extract and Vitamin C respectively for 14 days. Animals (200-250 g) were sacrificed by cervical dislocation at day 0, day 7 and Day 14 so as to assess the dose and time dependent effect of the extract. Findings revealed that OG leaf extract caused a dose-dependent significant protective effect ( $P < 0.05$ ) on the packed cell volume (PCV), total white blood cell (WBC) count, haemoglobin (Hb), platelet count and total red blood cell (RBC) count compared to Vitamin C treated group. Our study confirms that OG leaf extract is better at improving haematological parameters of restrain-stressed rats compared to Vitamin C and it was more effective in animals that were administered 100 mg/kg and 400 mg/kg of the extract. The result indicates that OG leaf extract could alleviate prolonged exposure to stress by lowering PCV, total WBC, total RBC, hemoglobin count and elevating blood platelets.

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Stress is a prime signal that re-orders homeostasis by continually interrupting the body's physiology (Bhattacharya and Ghosal, 2000). Several stress agents otherwise known as stressors trigger a "crisis-mode" physiological response, which the body attempts to return to homeostasis by means of an adaptive response (Calabrese *et al.*, 2007). Stress and its associated pathologies account for over 70% of illness globally (Bamidele *et al.*, 2020). Prolonged restrained stress (freedom barred stress) may lead to long-term pathologies of the cardiovascular system. Such stressors have the ability to disrupts the reproductive system as well as the normal function of hypothalamus pituitary- endocrine glands axes thus leading to changes in the levels of hormones (O'Connor *et al.*, 2020). Another form of stress called the "immobilization stress" enhances Nitric oxide synthase activity that led to alterations in levels of plasma as well as changes in human body physiology (Yaribeygi *et al.*, 2017). Aversive stimulus may result to motivation and improved task performance whereas distressing stimulus leads to anxiety, depression, social dysfunction and intentions of suicide (Bamidele

*et al.*, 2020). Birben *et al.* (2012) reported that stress increases the production of reactive oxygen species (ROS) and depletion of the antioxidant defense system associated with lipid peroxidation. However, Ramadan and Alshamrani (2015) demonstrated that stress disrupts haematological parameters in rats. Another study reported that stress alters the well-being of air breathing loaches by altering their hematological parameters (Kandeepan, 2014). To date, naturally occurring biological active constituents of several plants is used in traditional medicine due to their availability and low cost. This has led to a rapid increase in the use of these herbal remedies especially in the management of different diseases with several of them incorporated into orthodox medicine (Ekor, 2014; Erhirhie *et al.*, 2015; Anachuna *et al.*, 2018; Moke *et al.*, 2019; Bamidele *et al.*, 2020). The effect of herbal medicine on haematology have been reported in recent times (Emudainhwo *et al.*, 2015; Ladokun *et al.*, 2015; Moke *et al.*, 2018). Demand of plants containing essential oil in orthodox medicine is on the increase (Ueno *et al.*, 2019). One of such plants is *Ocimum gratissimum* (OG). It is a perennial

\*Corresponding Author Email: [goldasaba20@gmail.com](mailto:goldasaba20@gmail.com), Tel: +2348060410865

herbaceous plant cultivated in Nigeria due to its culinary and medicinal importance (Aguiyi *et al.*, 2000; Ofem *et al.*, 2012; Shittu *et al.*, 2016). Ijeh *et al.* (2004) demonstrated the presence of alkaloids, tannins, flavonoids, phytates, and oligosaccharides on phytochemical screening of OG. Its essential oil contains eugenol as one of its active ingredients and it was demonstrated to possess antibacterial activities (Ayinla *et al.*, 2011; Omodamiro and Jimoh, 2015). A study by Shittu *et al.* (2016) reported that OG administration improved blood parameters in diabetes mellitus experimental animal models. Studies by Ayinla *et al.*, (2011) and Chao *et al.*, (2016) revealed that OG consumption causes marked hypolipidaemic effect. Many synthetic drugs such as amphetamine, benzodiazepines, caffeine and anabolic steroids have been applied in the management of stress. A rise in the use of herbal medicine in developing regions have been reported (WHO, 2013; Ekor, 2014; Moke *et al.*, 2021). Sarjan *et al.* (2017) demonstrated a highly significant result on the use of an herbal extract on chronic stress induced immunodeficiency in rats. Owing to the numerous reported medicinal benefits of OG, the present study was designed to investigate the effect of ethanollic extract OG on haematological indices in stress-induced male Wistar rats.

## MATERIALS AND METHODS

**Experimental animals:** Thirty female Wistar rats, weighing between 200 – 250 g were purchased and housed in the animal house of College of Health Sciences, Delta State University Abraka, Nigeria. Animals were housed in propylene cages to allow adequate ventilation and their cages were cleaned daily. They were maintained under standard room temperature and 12 h light/dark cycle. The animals were acclimated for seven days and were provided with standard pellet diet and water given *ad libitum*. All experimental protocols were conducted according to the guiding principles of the Research Ethics on the Use of Laboratory Animals of the Delta State University Abraka with reference number (REC/FBMS/DELSU/19/81) as recommended by the declaration of Helsinki and the Guiding principles in the care and use of animals.

**Plant Materials:** Fresh leaves of OG plants were obtained from a farmland in Abraka, Ethiope East Local Government of Delta State of Nigeria. The plant was authenticated, and identified by Dr. A.H. Erhenhi in the Herbarium Unit of the Botany Department of Delta State University, Abraka.

**Plant extracts preparation:** Fresh leaves of OG were washed in running tap water, air-dried for three days before it was ground into a powdered form. Thereafter,

50 g of OG powder was soaked in 100 ml of ethanol for 24 hrs. The concentrate was kept inside the heating mantle of the fume cupboard of the extracting machine to evaporate the ethanol and the final product, which was a semi-solid residue, was stored in the refrigerator. The dose of OG administered was adopted from Shittu *et al.* (2016).

**Stress induction:** Model of chronic stress induction was adopted from Gudipudi *et al.* (2015). The rats were placed in plastic tubes (inner diameter, 5.7 cm; length, 20.3 cm) fixed with adhesive tape on the outside. One end of the tube remained open to allow breathing. The tubes completely restricted lateral movement and front to back movement of the animals. Immediately the animal enters inside the tube, it is placed horizontally to make the animal uncomfortable. The animals were exposed to stress for one hour in the morning between 8:00 am and 11:00 am daily.

**Experimental design:** Experimental animals were grouped into six groups containing five rats per group. The groupings were as follows: Group 1 (Normal control): No stress induction and rats fed normal diet. Group 2 (Restrained stress control – RS control): Stress was induced by retraining the rats for one hour with rats fed normal diet. Group 3 (RS+100 mg/kg): Stress was induced by restraining the rats for an hour and treated with 100 mg/kg/bw. Group 4 (RS+200 mg/kg) one hour restraint stress (RS) animals treated with 200 mg/kg/bw of OG leaf extract. Group 5 (RS+400 mg/kg): contained RS animals treated with 400 mg/kg/bw of OG leaf extract. Group 6 (standard group - RS+Vitamin C) one hour RS animals treated with 200 mg/kg/bw of Vitamin C. Treatments were administered orally daily for 14 days.

**Animal sacrifice and blood collection:** Animals (two rats per group) were sacrificed randomly at Day 0, Day 7 and Day 14 respectively in order to assess the dose and time dependent effect of OG in attenuating stress. Animals were sacrificed by cervical dislocation and blood was collected from the vena cava into well-labeled EDTA bottles.

**Haematological assessment:** Total red blood cell (Total RBC) count, packed cell volume (PCV), total white blood cell (Total WBC) count, were assessed using the Hemocytometer method (Thrall *et al.*, 2012). Hemoglobin (Hb) concentration was measured using the Cyanmethemoglobin method of Higgins (Thrall *et al.*, 2012).

**Statistical Analysis:** Data were subjected to Statistical Package for Social Sciences, version 23 (SPSS produced by SPSS Inc. Chicago), analyzed, and expressed as Mean  $\pm$  SEM (Standard Error of Mean).

Statistical comparisons were performed by One Way Analysis of Variance (ANOVA) followed by Fisher's Least Significant Difference (LSD) multiple mean comparison analysis. P values of < 0.05 were considered statistically significant.

## RESULTS AND DISCUSSION

The effect of *Ocimum gratissimum* on haematological parameters of Wistar rats under restrained stress is shown in Tables 1-5. There was significant decrease ( $P < 0.05$ ) in the PCV level of experimental animals treated with 100 mg/kg/bw and 400 mg/kg/bw of OG for 7 days and in all treated groups administered varying doses of OG for 14 days when compared with normal and RS control groups. Maximal significant decrease in PCV was seen in experimental animals that were administered 400 mg/kg/bw of OG. (Table 1). Table 2 shows significant decrease ( $P < 0.05$ ) in the hemoglobin level of RS experimental animals administered 100 mg/kg/bw and 400 mg/kg/bw of OG for 7 days when compared to the normal control and RS control. There was a non-significant decrease ( $P > 0.05$ ) in all RS experimental animals that received OG and vitamin C when compared with normal control and RS control groups. Highest significant decline in Hb level was obtained in RS animals administered 100 mg/kg/bw of OG for 7 days. (Table

2). Restrained stress animals administered OG shows significant ( $P < 0.05$ ) decrease in total RBC in the experimental rats administered 100 mg/kg/bw and 200 mg/kg/bw of OG for 7 days when compared with the normal and RS control groups. However, there is a non-significant ( $P > 0.05$ ) decrease in total RBC count following the administration of varying doses of OG and vitamin C for 14 days. (Table 3). Total WBC was decrease in the experimental animals treated with varying doses of OG for 7 and 14 days when compared with the normal and RS controls. Animals treated with vitamin C had a higher total WBC count as compared to the RS control.

However, no observations were significant. (Table 4). Seven (7) days treatment showed non-significant ( $P > 0.05$ ) difference in RS rats administered vitamin C for 7 days when compared with the normal and RS controls. Only RS animals that received 200 mg/kg/bw of OG for 7 days showed a significant ( $P < 0.05$ ) decrease in the platelet compared to the normal control and RS groups. There was non-significant ( $P > 0.05$ ) increase in platelet count of RS experimental rats administered OG and vitamin C for 14 days. However, significant increase in platelet count was shown in RS animals that received 100 mg/kg/bw of OG and vitamin C respectively for 7 days and in RS rats administered vitamin C for 14 days.

**Table 1:** Effect of *Ocimum gratissimum* on packed cell volume (%) of Wistar rats under restrained stress

Groups	Day 0	Day 7	Day 14
Normal Control	38.33 ± 1.09	43.00 ± 2.15	38.00 ± 2.03
RS Control	36.33 ± 3.42	37.00 ± 1.14	39.00 ± 1.48
RS+100 mg/kg	35.63 ± 1.13	29.33 ± 2.17 <sup>#</sup>	33.75 ± 3.40 <sup>#</sup>
RS+200 mg/kg	37.33 ± 2.07	38.25 ± 3.10	31.67 ± 2.07 <sup>#</sup>
RS+400 mg/kg	34.33 ± 1.31	26.00 ± 2.12 <sup>#</sup>	28.25 ± 3.95 <sup>#</sup>
RS+Vitamin C	38.33 ± 2.09	39.00 ± 2.15	34.50 ± 2.03

All values are expressed as mean ± standard error of mean (SEM); \* =  $P < 0.05$  when compared with normal control; # =  $P < 0.05$  when compared with restrained stress (RS) control.

**Table 2:** Effect of *Ocimum gratissimum* on hemoglobin (Hb) concentration (g/dl) of Wistar rats under restrained stress

Groups	Day 0	Day 7	Day 14
Normal Control	11.78 ± 0.69	14.33 ± 1.15	12.33 ± 2.03
RS Control	12.18 ± 0.12	14.83 ± 0.14	12.75 ± 1.48
RS+100 mg/kg	10.78 ± 0.13	7.56 ± 0.17 <sup>#</sup>	11.25 ± 1.22
RS+200 mg/kg	12.78 ± 0.17	12.75 ± 1.10	10.56 ± 2.00
RS+400 mg/kg	11.78 ± 1.31	8.66 ± 0.12 <sup>#</sup>	9.42 ± 1.95
RS+Vitamin C	12.48 ± 0.09	13.00 ± 0.15	11.75 ± 1.56

All values are expressed as mean ± standard error of mean (SEM); \* =  $P < 0.05$  when compared with normal control; # =  $P < 0.05$  when compared with restrained stress (RS) control.

**Table 3:** Effect of *Ocimum gratissimum* on total red blood cell count ( $\times 10^9/L$ ) of Wistar rats under restrained stress

Groups	Day 0	Day 7	Day 14
Normal Control	3.91 ± 0.09	4.18 ± 0.15	4.03 ± 2.03
RS Control	3.71 ± 0.42	4.67 ± 0.14	4.17 ± 1.48
RS+100 mg/kg	3.22 ± 0.13	3.44 ± 0.17 <sup>#</sup>	4.12 ± 1.40
RS+200 mg/kg	3.62 ± 0.07	3.47 ± 0.10 <sup>#</sup>	3.61 ± 2.07
RS+400 mg/kg	3.59 ± 0.31	3.83 ± 0.12	3.70 ± 1.95
RS+Vitamin C	3.91 ± 0.09	3.92 ± 0.15	3.95 ± 2.03

All values are expressed as mean ± standard error of mean (SEM); \* =  $P < 0.05$  when compared with normal control; # =  $P < 0.05$  when compared with restrained stress (RS) control.

**Table 4:** Effect of *Ocimum gratissimum* on total white blood cell count ( $\times 10^9/L$ ) of Wistar rats under restrained stress

Groups	Day 0	Day 7	Day 14
Normal Control	8.27 $\pm$ 1.12	10.20 $\pm$ 0.65	10.80 $\pm$ 1.33
RS Control	8.07 $\pm$ 0.12	11.22 $\pm$ 1.44	11.70 $\pm$ 1.28
RS+100 mg/kg	6.77 $\pm$ 1.28	8.26 $\pm$ 2.17	9.90 $\pm$ 0.40
RS+200 mg/kg	7.77 $\pm$ 0.27	9.30 $\pm$ 0.30	8.67 $\pm$ 1.07
RS+400 mg/kg	6.77 $\pm$ 0.34	9.20 $\pm$ 1.32	8.90 $\pm$ 1.48
RS+Vitamin C	8.04 $\pm$ 1.46	10.80 $\pm$ 1.16	10.20 $\pm$ 2.00

All values are expressed as mean  $\pm$  standard error of mean (SEM); \* =  $P < 0.05$  when compared with normal control; # =  $P < 0.05$  when compared with restrained stress (RS) control.

**Table 5:** Effect of *Ocimum gratissimum* on platelet count ( $\times 10^9/L$ ) of Wistar rats under restrained stress

Groups	Day 0	Day 7	Day 14
Normal Control	131.67 $\pm$ 9.09	162.55 $\pm$ 8.15	128.33 $\pm$ 9.03
RS Control	121.67 $\pm$ 8.42	125.50 $\pm$ 8.14	125.00 $\pm$ 7.48
RS+100 mg/kg	137.67 $\pm$ 8.13	173.33 $\pm$ 9.17*#	125.50 $\pm$ 9.40
RS+200 mg/kg	133.67 $\pm$ 6.07	157.50 $\pm$ 3.10*#	133.33 $\pm$ 7.07
RS+400 mg/kg	118.67 $\pm$ 4.31	135.00 $\pm$ 5.12	130.50 $\pm$ 3.95
RS+Vitamin C	128.67 $\pm$ 8.09	173.35 $\pm$ 5.15*#	157.50 $\pm$ 7.03*#

All values are expressed as mean  $\pm$  standard error of mean (SEM); \* =  $P < 0.05$  when compared with normal control; # =  $P < 0.05$  when compared with restrained stress (RS) control.

The present study evaluated the beneficial effect of ethanollic extract of *Ocimum gratissimum* (OG) on haematological parameters of adult female Wistar rats under restrained stress. Restrained stress is reported to alter the body homeostasis and contributes to cardiovascular pathologies (Black and Garbutt, 2002). It has the ability of interrupting the functions of neuroendocrine cells and releasing hormones of the hypothalamus (O'Connor *et al.*, 2020). Consequently, hematological parameters have been proven as viable markers for the assessment of health in humans and animals and several studies have focused on hematological indices as an indicator for stress evaluation (Kandeepan, 2014; Kori *et al.*, 2017, Pandian *et al.*, 2018; Ueno *et al.*, 2019; Bamidele *et al.*, 2020). Results from our study recorded an increase in PCV and total RBC in the stressed groups. However, following administration with OG extract, we recorded a dose-dependent significant increase in PCV and total RBC compared to vitamin C that is a standard antioxidant. This implies that extract of OG alters stress-induced polycythemia and erythrocytosis and confirms that OG leaf extract is more potent in alleviating stress than vitamin C. The decline might be linked to the antioxidative activity of OG through its total phenolic content (Ofem *et al.*, 2012; Kori *et al.*, 2017; Okechukwu *et al.*, 2019). Reasons for this observation may be attributed to the fact that tissue anoxia causes the production of erythropoietin, which enhances erythrocyte production (Bowman *et al.*, 2009). Consequently, Eburnlomo *et al.* (2012) demonstrated that stress induces a no significant change in RBC. White blood cell (WBC) counts are the first line indicators of immune response and they are the earliest blood cells to respond to immune modulating agents and stressors (Eburnlomo *et al.*, 2012). We hypothesized that OG and vitamin C may alter total WBC count of RS Wistar rats. In this study,

100, 200, and 400 mg/kg/bw of OC and vitamin C were administered to restrained animals for 7 and 14 days. Our study demonstrated that stress elevates WBC counts. This increase in WBC is in tandem with a study by Bamidele *et al.* (2020) who reported that stress increases circulating WBCs in the blood but disagrees with that of Ramadan and Alshamrani (2015) who reported a decline in WBC of animals exposed to immobilized stress. During stress, the hypothalamus secretes stress hormones (corticotropin-releasing hormone) which acts on the pituitary gland to initiate regulated stress response pathway by activating the release of immune boosters such as WBC (O'Connor *et al.*, 2020). More so, increase WBCs may be linked to increase beta-adrenergic binding during chronic stress (Abiramasudari *et al.*, 2012). Interestingly, our study revealed that administration of OG extract to immobilized rats led to a decline in stress-induced increase in WBC compared to vitamin C. Although the exact mechanism of action of OG in reducing WBC in stressed animals is unknown, it is believed that plants with antioxidants reduce blood glucose by inhibiting the breakdown of glucose (Bamidele *et al.*, 2020). Also, the reduction in WBC may also be a proof of the plants antibacterial activities as previously reported (Matasyoh *et al.*, 2007; Omodamiro and Jimoh, 2015).

A significant dose-dependent increase in haemoglobin concentration in OG treated female RS rats was observed when compared to the normal control and stress control animal, thus indicating that OG increases the concentration of haemoglobin. The significant increase in hemoglobin concentration is linked to the various constituents of OG such as calcium, potassium, magnesium, phosphorous, iron, zinc, manganese, sodium, nickel, copper, and

vitamins, which are necessary for the formation of blood cells (Asaolu *et al.*, 2012).

Platelets play major functions in the maintenance of body milieu (Cognasse *et al.*, 2019). Blood platelets, mediate inflammatory responses and functions in the effective clearance of pathogens (Cognasse *et al.*, 2019). Our study demonstrated a significant decrease in platelet counts in restrained animals when compared to the control and experimental groups. One of the mechanisms behind this observation may be that stress is reported to activate the sympathetic part of the nervous system thus leading to an elevation of catecholamines, norepinephrine and epinephrine (Von Känel and Dimsdale, 2000; Brydon and Magid, 2006). Another study showed that epinephrine and norepinephrine enhance activation of blood platelet (Von Känel and Dimsdale, 2000) while Steptoe *et al.* (2003) linked platelet activation and stress-induced cardiovascular responses to the sympathetic nervous system. Our study revealed a significant increase in platelet count of the experimental animals following OG and vitamin C administration compared to the normal control and RS control groups. This result pattern disagrees with the findings by Ofem *et al.* (2012) who demonstrated that oral administration of OG to male albino rats led to decline in platelet indices although in their study experimental animals were not subjected to stress. This observation may be because of the interactions between the antioxidative properties of OG leaf and the  $\alpha$ -adrenergic receptors and  $\alpha$ -adrenergic receptors in surface of the platelet membranes or it could be that eugenol an essential oil of OG activates the production and expression of thrombopoietin. Even though the mechanisms of action of co-administration of OG on prolonged stress is unknown, our result findings indicated that oral administration of OG could relieve some hematological stress indices. This may be attributed to the antioxidative properties of OG (Okechukwu *et al.*, 2019).

**Conclusion:** Our study showed that intake of *Ocimum gratissimum* is effective in alleviating prolonged stress. *Ocimum gratissimum* leaves extract demonstrated promising results in combating polycythemia, erythrocytosis, and thrombocytopenia induced by exposure to stress. Hence, consumption of *Ocimum gratissimum* leaves as part of human dietary supplement is advised.

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