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FUTA Journal of Research in Sciences

ISSN: 2315-8239

FUTA Journal of Research in Sciences, 2014 (2): 157-169

IMMUNOSTIMULATORY EFFECTS OF ETHANOLIC LEAF EXTRACT OF SWEET BASIL (*OCIMUM GRATISSIMUM)* ON WISTAR RATS INFECTED WITH *SALMONELLA TYPHI*

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ABSTRACT

Sweet basil (*Ocimum gratissimum*) is widely consumed as green leafy vegetable and used in folkoric medicine in the treatment and management of both infectious and non-infectious diseases. This study was designed to investigate the effect of ethanolic leaf extract of *O. gratissimum* on the immune system of Wistar rats infected with *Salmonella typhi*. Forty Wistar rats were divided into eight groups of five rats each. Groups 1, 2A, and 2B were infected with *Salmonella typhi* and observed for signs of infection before being treated with 200mg/kg and 400mg/kg extract of *O. gratissimum;* Groups 3A and 3B were fed with 200mg/kg and 400mg/kg extract of *O. gratissimum* respectively in addition to their normal diet; Group 4 was infected with *S. typhi* and left untreated; Group 5 was infected with *S. typhi*, observed for signs of infection and later treated with 250mg/kg of ciprofloxacin; while Group 6 was fed basal diet and water only. Blood and urine were collected from the rats at the end of the experiment. Protein was present in all the urine examined before, during, and after infection, while glucose, blood, and ketone were absent. The urine collected before and during infection contained crystals (majorly triple phosphate) and bacteria when examined microscopically. Apparently, *O. gratissimum* did not affect metabolic activities of the experimental Wistar rats. White blood cells and RBCs in the groups pre-treated with the extract did not differ significantly; hence the use of ethanolic leaf extract of *O. gratissimum* as prophylactics is not encouraged. Compared to ciprofloxacin, the ethanolic extract at 200mg/kg significantly ($p<0.05$) increased the production of RBCs. However, production of neutrophils and lymphocytes which are essential immune cells did not differ significantly ($p<0.05$) between groups. Resultantly, this study revealed that ethanolic leaf extract of *O. gratissimum* may enhance erythropoiesis but not immunostimulation during *S. typhi* infection.

Keywords: *Ocimum gratissimum, Salmonella typhi,* immune system, Wistar rats, ethanolic extract

INTRODUCTION

Ocimum gratissimum, popularly known as scent leaf because of its aromatic odour, is an herbaceous plant with woody base. It contains various phytochemicals which perform many functions in plant and have prominent effect on animal systems and microbial cells (Awe and Omojasola, 2009; Akande *et al*, 2010). Various studies have revealed numerous potentials of the plant including therapeutic activity (Ijeh *et al*, 2005; Adebolu and Salau, 2005), antiviral and anti-parasitic activities (Holetz *et al*, 2003; Ayisi and Nyadedzor, 2003), healing of wounds and dermatological infections (Orafidiya *et al*, 2005), anti-convulsant and anti-oxidative activities (Aprioku and Obianime, 2008; Okoli *et al*, 2010; Abdulazeez *et al*, 2013), sexual

invigoration (Pande and Pathak, 2009), insecticidal activity (Brisibe *et al*, 2011), culinary purpose (Okoli *et al*, 2010), crop protection (Amadi *et al*, 2010), and antibacterial activity (Nakamura *et al*, 1999). In this regard, *O. gratissimum* is one of the widely consumed botanicals in developing countries, especially Africa.

Human is constantly exposed to pathogens through his interactions with the environment. *Salmonella typhi*, the causative agent of typhoid, is an example of pathogens transmitted to man through ingestion of contaminated food and/or water (Okafor, 2007). Infective dose as few as 10^3 of *S. typhi* are capable of causing infections in humans when ingested. The ingested bacteria attach to the cells of the jejunum (M cells) and invade the cells by means of endocytosis, transfer, and exocytosis. They are phagocytosed by macrophages in the sub-serosa and translocated into the mesenteric lymph node where proliferation occurs (Kayser *et al*, 2005). The bacilli reach the bloodstream principally by lymph drainage from mesenteric nodes to the thoracic duct and general circulation (WHO, 2003). Systemic dissemination of the organisms to the reticuloendothelial system (liver, spleen, bone marrow, etc) occurs and for a period of 1-3 weeks, the bacteria multiply within these organs. Subsequently, the infected cells of the organs rupture and release the bacteria into the bile. They re-infect the lymphoid tissue of the small intestine particularly in the ileum while the organisms that do not re-infect the host are excreted in stool (WHO, 2003). Typhoid fever is a global health problem with approximately 17 million cases and 600,000 deaths occurring annually. Usually, its clinical picture is confused with those of many other febrile infections and the rate of transmission is high especially in individuals between 3 and 19 years of age and in regions such as Central Asia, Latin America, Vietnam, Indonesia, and Sub-Saharan Africa where overcrowding, poor sanitary conditions, and untreated water supplies prevails (WHO, 2003).

The immune system protects the body against invading pathogens by generating several cells and molecules which eliminate the invaders (Dashputre and Naikwade, 2010). Immunomodulators are being used as supportive adjunct to specific antibiotic therapy in immunodeficient patients (Kayser *et al*, 2005). Studies have also shown that some herbs are capable of stimulating the immune system; especially the innate immunity and this could be beneficial in immunotherapy and also serve as alternative to conventional chemotherapy (Dashputre and Naikwade, 2010; Ashoka Shenoy *et al*, 2009; Oladunmoye, 2006). However, with respect to *O. gratissimum*, very little work has been done to determine its effect on the immune system of Wistar rats especially when infected with *S. typhi*. In view of the above, this study was designed to investigate the immunostimulatory effects of ethanolic leaf extract of sweet basil (*O. gratissimum*) on Wistar rats infected with *S. typhi*.

MATERIALS AND METHODS

Collection, preparation and extraction of plant material

Plants of *Ocimum gratissimum* were collected from the premises of the Federal School of Agriculture, Akure, Nigeria. According to Oladunmoye (2006) and Nwinyi *et al* (2009), the leaves were removed and dried in the shade for two weeks before being pulverized into fine powder using Marlex Excella mixer grinder and kept in an air-tight container. One hundred and fifty gram (150g) of the pulverized leaves was soaked in 1500ml of 70% ethanol for 72 hours. The preparation was later sieved with clean muslin cloth to obtain filtrate which was concentrated using rotary evaporator and then freeze dried.

Test Bacteria

Stock culture of *S. typhi* used in this study was collected from the State Specialist Hospital, Akure, Ondo State, Nigeria on Tryptone Soy Agar (TSA) slant.

Laboratory Animals Used

The Wistar rats used for this study were obtained from the Animal House of the Multi-disciplinary Laboratory, Obafemi Awolowo University, Ile Ife, Osun State, Nigeria and acclimatized for seven days. For the purpose of

this experiment, thirty rats were used to determine the infective dose 50 (ID_{50}) of *S. typhi* while forty rats were divided into eight groups of five rats each to study the effect of ethanolic leaf extract of *O. gratissimum* in the animals.

Preparation of Inoculum Culture of *S. typhi* for *in vivo* Assay and Determination of Infective Dose 50 (ID_{50})

Re-activation of *S. typhi* from the stock culture, preparation of the bacterial inoculum culture for *in-vivo* assay and determination of the infective dose 50 were performed as described by Takumi *et al* (2002). The test bacteria were cultured in Tryptone Soy Broth and incubated at 37^0C for 24 hours. The culture was then centrifuged at 2000 rpm for 10 minutes. The supernatant was discarded and the pellet was re-suspended in 100ml physiological saline, followed by re-centrifugation. Finally, the pellet was re-suspended in 4ml physiological saline and the cell suspension was serially diluted. Infective dose-50 for *S. typhi* was determined by oral inoculation of thirty Wistar rats, divided into six groups, with the bacterial dilutions mixed with equal volume of 6% (w/v) $NaHCO_3$. Also, corresponding CFU/ml was determined for each bacterial dilution in the laboratory using plate count method on Salmonella-Shigella Agar (SSA)

Evaluation of the Immunostimulatory Effects of Ethanolic Leaf Extract of *O. gratissimum* on Wistar Rats

Forty Wistar rats were divided into eight groups to study the immunostimulatory potential of ethanolic leaf extract of *O. gratissimum*. Group 1 was orally inoculated with the infective dose of *S. typhi* which was calculated to be 2.20×10^5 CFU/ml and observed for signs of infection before being treated with 200mg/kg ethanolic leaf extract of *O. gratissimum*. Groups 2A and 2B were given prophylactic dose of 200mg/kg and 400mg/kg extract of *O. gratissimum* respectively before being infected with *S. typhi* and later treated with the same doses of the extract; Groups 3A and 3B were fed with 200mg/kg and 400mg/kg extract of *O. gratissimum* respectively for three weeks; Group 4 was infected and left untreated; Group 5 was infected with *S. typhi* and then treated with 250mg/kg of ciprofloxacin; while Group 6 was given water and basal diet only (control).

Collection and examination of urine and blood of the Wistar rats

Urine was collected from the experimental rats for urinalysis using Medi-Test Combi 10 SGL test strips from Macherey-Nagel GmbH & Co, Germany. Again, microscopy of the urine was done as described by Maiti (2010). The rats were sacrificed and their blood, collected into labeled EDTA bottles, was analysed using autoanalyser Cyflow SL-3 manufactured by Partec.

Statistical Analysis

The data obtained were analyzed using one-way analysis variance (ANOVA) and presented as mean ± standard deviation (SD). The level of significance was considered at $p < 0.05$.

RESULTS

Effects of Ethanolic Leaf Extract of *O. gratissimum* on Urine Parameters of the Wistar Rats

All the urine collected before infection was negative for glucose, ketone, blood, and normal for urobilinogen. The pH was 7.0, the density ranged between 1.010 and 1.015; bilirubin was moderately present in the urine of the rats fed with 200mg/kg extract in addition to their basal meal and of the rats fed with basal meal only (Table 1). During infection, all the urine was negative for blood, ketone, glucose, and normal for urobilinogen while the density and pH were 1.000 and 8.0 respectively (Table 2). After infection, the pH of the urine from the rats treated with the extract of *O. gratissimum* was 8.0 and 6.0 for the rats treated with ciprofloxacin. Bilirubin was negative and the density ranged between 1.005 and 1.025 (Table 3). The urine collected from all the groups before, during and after infection was positive for protein. Also, microscopy of the urine revealed the presence of crystals (majorly triple phosphates) (Table 4). After infection, the urine contained bacteria, amorphous phosphate granules, pus cells, and casts as shown in Table 5.

Effects of Ethanolic Leaf Extract of *O. gratissimum* on Haematological Parameters of Wistar Rats

In Table 6, rats fed with 400mg extract/kg body weight had the highest mean value of packed cell volume (PCV) (47.6%) while the lowest mean value of haemoglobin (Hb), 12.7g/dl, was seen in the group given prophylactic treatment with 400mg extract/kg body weight. In comparison with the group treated with ciprofloxacin, RBCs increased significantly (p-value of 0.001) in the group treated with 200mg extract (G1) while mean RBCs values significantly (p-value of 0.007) differ between the ciprofloxacin-treated group (G5) and infected-untreated group (G4). Moreover, between the infected-untreated group (G4) and the group pre-treated with 200mg extract, mean corpuscular haemoglobin (MCH) differ significantly ($p<0.05$). As revealed in Table 7, production of neutrophils and lymphocytes did not differ significantly between groups. However, in comparison with the control (G6), total WBC counts reduced significantly ($P<0.05$) in the ciprofloxacin-treated group (G5) and the group pre-treated with 200mg extract of *O. gratissimum* (G2A).

Table 1: Effects of Ethanolic Leaf Extract of *O. gratissimum* on Urine Parameters of Wistar Rats before Infection

Parameters	Experimental groups							
	G1	G2A	G2B	G3A	G3B	G4	G5	G6
Blood	-	-	-	-	-	-	-	-
Urobilinogen	N	N	N	N	N	N	N	N
Bilirubin	-	-	-	+	-	-	-	+
Protein	+	+	+	+	+	+	+	+
Nitrite	-	-	P	-	P	-	-	P
Ketone	-	-	-	-	-	-	-	-
Glucose	-	-	-	-	-	-	-	-
P^H	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0
Density	1.010	1.015	1.010	1.010	1.010	1.015	1.010	1.010
Leucocytes	-	-	-	-	-	-	-	-

Keys:

N = Normal; P = Positive; - = Negative; + = Present; **G1** = Rats infected with *S. typhi* and treated; **G2A** = Rats pre-treated with 200mg extract/kg body weight before infection; **G2B** = Rats pre-treated with 400mg extract/kg body weight before infection; **G3A** = Rats fed with 200mg extract/kg body weight in addition to their basal diet; **G3B** = Rats fed with 400mg extract/kg body weight in addition to their basal diet; **G4** = Rats infected but not treated with the extract; **G5** = Rats infected and treated with ciprofloxacin; **G6** = Rats fed with normal diets and water only (control)

Table 2: Effects of Ethanolic Leaf Extract of *O. gratissimum* on Urine Parameters of Wistar Rats during Infection

Parameters	Experimental groups							
	G1	**G2A**	**G2B**	**G3A**	**G3B**	**G4**	**G5**	**G6**
Blood	-	-	-	-	-	-	-	-
Urobilinogen	N	N	N	N	N	N	N	N
Bilirubin	+	-	-	-	-	-	-	+
Protein	+	+	+	+	+	+	+	+
Nitrite	-	-	P	-	P	-	-	P
Ketone	-	-	-	-	-	-	-	-
Glucose	-	-	-	-	-	-	-	-
P^H	8.0	8.0	8.0	8.0	8.0	8.0	8.0	7.0
Density	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.010
Leucocytes	-	-	-	Ca 25	Ca 25	Ca 25	-	-

Keys:

N = Normal; P = Positive; - = Negative; + = Present; **G1** = Rats infected with *S. typhi* and treated; **G2A** = Rats pre-treated with 200mg extract/kg body weight before infection; **G2B** = Rats pre-treated with 400mg extract/kg body weight before infection; **G3A** = Rats fed with 200mg extract/kg body weight in addition to their basal diet; **G3B** = Rats fed with 400mg extract/kg body weight in addition to their basal diet; **G4** = Rats infected but not treated with the extract; **G5** = Rats infected and treated with ciprofloxacin; **G6** = Rats fed with normal diets and water only (control)

Table 3: Effects of Ethanolic Leaf Extract of *O. gratissimum* on Urine Parameters of Wistar Rats after Infection

Parameters	Experimental groups							
	G1	G2A	G2B	G3A	G3B	G4	G5	G6
Blood	-	-	-	-	-	-	-	-
Urobilinogen	N	N	N	N	N	N	N	N
Bilirubin	+	+	+	+	+	+	+	+
Protein	+	+	+	+	+	+	+	+
Nitrite	-	-	-	-	-	-	-	-
Ketone	-	-	-	-	-	-	-	-
Glucose	-	-	-	-	-	-	-	-
P^H	8.0	8.0	8.0	8.0	8.0	8.0	6.0	7.0
Density	1.005	1.000	1.000	1.000	1.000	1.000	1.025	1.010
Leucocytes	-	-	-	-	-	-	-	-

Keys:

N = Normal; - = Negative; + = Present; **G1** = Rats infected with *S. typhi* and treated; **G2A** = Rats pre-treated with 200mg extract/kg body weight before infection; **G2B** = Rats pre-treated with 400mg extract/kg body weight before infection; **G3A** = Rats fed with 200mg extract/kg body weight in addition to their basal diet; **G3B** = Rats fed with 400mg extract/kg body weight in addition to their basal diet; **G4** = Rats infected but not treated with the extract; **G5** = Rats infected and treated with ciprofloxacin; **G6** = Rats fed with normal diets and water only (control)

Table 4: Microscopy of the Rat's Urine before Infection

Parameters	Experimental groups							
	G1	**G2A**	**G2B**	**G3A**	**G3B**	**G4**	**G5**	**G6**
Appearance	Y/ST	Y/ST	Y/ST	Y/ST	Y/ST	Y/ST	Y/ST	Y/ST
Crystals	TP	TP	TP	TP	TP	TP	TP	TP, CO
Casts	NS	NS	NS	NS	NS	NS	NS	NS
Bacteria	S	S	S	S	S	S	S	S

Keys:

Y = Yellow; ST = Slightly turbid; NS = Not seen; S = Seen; TP = Triple phosphate crystals; CO = Calcium oxalate crystals; **G1** = Rats infected with *S. typhi* and treated; **G2A** = Rats pre-treated with 200mg extract/kg body weight before infection; **G2B** = Rats pre-treated with 400mg extract/kg body weight before infection; **G3A** = Rats fed with 200mg extract/kg body weight in addition to their basal diet; **G3B** = Rats fed with 400mg extract/kg body weight in addition to their basal diet; **G4** = Rats infected but not treated with the extract; **G5** = Rats infected and treated with ciprofloxacin; **G6** = Rats fed with normal diets and water only (control)

Table 5: Microscopy of the Rat's Urine during Infection

Parameters	Experimental groups							
	G1	**G2A**	**G2B**	**G3A**	**G3B**	**G4**	**G5**	**G6**
Appearance	A/ST	A/ST	A/ST	A/ST	A/ST	A/ST	A/ST	Y/ST
Crystals	TP	TP	TP	TP, CO	TP	TP	TP	TP, CO
Bacteria	N	N	N	N	N	N	N	N
Casts	NS	NS	NS	S	NS	NS	NS	NS
Pus cells /HPF	0-2	0-1	0-1	NS	NS	0-2	0-1	NS
Amorphous phosphate granules	S	NS	NS	NS	NS	NS	NS	NS

Keys:

Y = Yellow; ST = Slightly turbid; NS = Not seen; S = Seen; TP = Triple phosphate crystals; CO = Calcium oxalate crystals; **G1** = Rats infected with *S. typhi* and treated; **G2A** = Rats pre-treated with 200mg extract/kg body weight before infection; **G2B** = Rats pre-treated with 400mg extract/kg body weight before infection; **G3A** = Rats fed with 200mg extract/kg body weight in addition to their basal diet; **G3B** = Rats fed with 400mg extract/kg body weight in addition to their basal diet; **G4** = Rats infected but not treated with the extract; **G5** = Rats infected and treated with ciprofloxacin; **G6** = Rats fed with normal diets and water only (control).

Table 6: Effects of ethanolic leaf extract of *Ocimum gratissimum* on PCV, Hb, and other haematological parameters of Wistar rats

Parameters	Experimental groups							
	G1 Mean ± SD	G2A Mean ± SD	G2B Mean ± SD	G3A Mean ± SD	G3B Mean ± SD	G4 Mean ± SD	G5 Mean ± SD	G6 Mean ± SD
PCV (%)	45.4 ± 1.88[a]	45.3 ± 2.98[a]	42.1 ± 5.98[a]	42.9 ± 4.48[a]	47.6 ± 3.65[a]	42.2 ± 1.8[a]	43.0 ± 4.52[a]	43.2 ± 1.00[a]
Hb (g/dL)	13.6 ± 0.59[a]	13.4 ± 0.82[a]	12.7 ± 1.19[a]	13.4 ± 0.83[a]	14.1 ± 0.92[a]	12.1 ± 0.5[a]	12.3 ± 1.28[a]	13.4 ± 0.47[a]
RBC ($X10^6$/µl)	7.8 ± 0.23[ab]	7.8 ± 0.58[ac]	7.2 ± 0.88[a]	7.1 ± 0.64[a]	8.0 ± 0.52[a]	6.5 ± 0.4[a]	6.6 ± 0.95[b]	7.1 ± 0.09[a]
MCHC (%)	30.0 ± 0.18[a]	29.6 ± 1.00[a]	30.3 ± 1.68[a]	29.9 ± 1.03[a]	29.6 ± 0.55[a]	28.6 ± 0.7[a]	30.7 ± 1.58[a]	31.0 ± 0.51[a]
MCH (pg)	17.4 ± 0.59[a]	17.2 ±0.39[ab]	17.6 ±0.75[a]	18.0±0.43[ab]	17.5±0.24[ab]	18.5 ± 1.1[a]	18.5 ± 0.89[a]	18.8 ± 0.49[a]
MCV	58.3 ± 2.23[a]	58.1 ± 3.05[a]	58.1 ± 1.31[a]	60.1 ± 1.68[a]	59.2 ± 1.09[a]	65.0 ± 5.4[a]	60.1 ± 2.89[a]	60.7 ± 0.70[a]
Platelets	44.7 ± 6.72[a]	146.3 ±7.09[a]	116.6 ±6.68[a]	137.0 ±6.08[a]	70.08 ±7.76[a]	285.9 ± 9.3[a]	252.9 ±7.54[a]	56.2 ± 7.74[a]

* Values with the same superscripts are not statistically different while those with dissimilar superscripts are significantly different

Keys:
PCV = Packed cell volume; **Hb** = Haemoglobin; **G1** = Rats infected with *S. typhi* and treated; **G2A** = Rats pre-treated with 200mg extract/kg body weight before infection; **G2B** = Rats pre-treated with 400mg extract/kg body weight before infection; **G3A** = Rats fed with 200mg extract/kg body weight in addition to their basal diet; **G3B** = Rats fed with 400mg extract/kg body weight in addition to their basal diet; **G4** = Rats infected but not treated with the extract; **G5** = Rats infected and treated with ciprofloxacin; **G6** = Rats fed with normal diets and water only (control)

Table 7: Effects of ethanolic leaf extract of *O. gratissimum* on total and differential WBC counts of Wistar rats

Experimental Groups	Relative values		Total WBC ($X10^3$ mm^3)	Absolute values	
	Neutrophils (%)	Lymphocytes (%)		Neutrophils (neutrophils/µl)	Lymphocytes (lymphocytes/µl)
G1	27.0 ± 10.74 [a]	73.0 ± 10.70 [a]	6.7 ± 4.54 [a]	180.9	489.1
G2A	22.0 ± 9.20 [a]	78.0 ± 9.20 [a]	5.8 ± 0.86 [ac]	127.6	452.4
G2B	19.2 ± 10.99 [a]	80.7 ± 10.99 [a]	7.0 ± 2.11 [a]	134.4	564.9
G3A	25.2 ± 9.29 [a]	74.7 ± 9.29 [a]	9.6 ± 3.30 [a]	241.9	717.1
G3B	35.5 ± 5.07 [a]	64.5 ± 5.07 [a]	8.8 ± 4.17 [a]	312.4	567.6
G4	27.6 ±18.3 [a]	72.3 ± 18.3 [a]	6.9 ± 1.18 [ab]	190.4	498.9
G5	29.5 ± 14.98 [a]	70.5 ± 14.98 [a]	10.5 ± 3.47 [a]	309.8	740.3
G6	13.0 ± 5.57 [a]	87.0 ± 5.57 [a]	7.6 ± 4.45 [a]	98.8	661.2

*****Absolute values of differential WBCs = Relative differential WBC value X Total WBC count

* Values with the same superscripts are not statistically different while those with dissimilar superscripts are significantly different

Keys:

G1 = Rats infected with *S. typhi* and treated; **G2A** = Rats pre-treated with 200mg extract/kg body weight before infection; **G2B** = Rats pre-treated with 400mg extract/kg body weight before infection; **G3A** = Rats fed with 200mg extract/kg body weight in addition to their basal diet; **G3B** = Rats fed with 400mg extract/kg body weight in addition to their basal diet; **G4** = Rats infected but not treated with the extract; **G5** = Rats infected and treated with ciprofloxacin; **G6** = Rats fed with normal diets and water only (control)

DISCUSSION

The protein observed in the urine of all the rats before, during and after infection could be major urinary proteins (Mups) which are present in abundance in the urine of rodents (Frohlich *et al*, 1984; Gordon *et al*, 1993) while the crystals found in the urine might be formed from the protein since urinary proteins play an important role in the formation of precipitate such as crystals (Tannehill-Greg *et al*, 2009). Apparently, *O. gratissimum* did not affect metabolic activities of the experimental Wistar rats. Unlike the ciprofloxacin-treated rats, production of red blood cells was significantly increased in the group treated with 200mg (G1) and the control group (G6) (p-values of 0.001, < 0.001). According to Colino *et al* (1998), erythrocytes membranes are highly permeable to quinolone and this might be responsible for the decrease observed in the RBCs of the ciprofloxacin-treated group. Furthermore, rats that were treated with 200mg extract (G1) demonstrated significant (p-value of 0.004) increase in RBC relative to the control group (G6). However, the packed cell volume (PCV) of the two groups did not differ proportionately with their RBCs. Similar observation was made by Opara *et al* (2012) while Effraim *et al* (2013) reported that the diuretic activity of *O. gratissimum* could result in slight increase in RBC count. Usually, typhoid fever is characterized by normal or low white blood cells count (Brooks *et al.,* 2007). This confirms the significant reduction (p-value of 0.002) in the values of total WBCs count of the infected-untreated rats (G4) when compared with control group (G6). In addition, bacterial activities during infection could damage the blood cells or blood forming tissues (Nwankpa *et al.,* 2014). The haematological parameters of the group treated with 200mg extract and that of the rats pre-treated with the same amount of the extract did not differ significantly. Thus, the use of ethanolic leaf extract of *O. gratissimum* as prophylactics is not encouraged. In conclusion, ethanolic leaf extract of *O. gratissimum* did not apparently affect urine parameters of the rats but prevents anaemia which is a major side effect of some of the drugs used for the treatment of typhoid. However, the amount of neutrophils and lymphocytes which are essential immune cells observed in this study did not differ significantly and the extract of *O. gratissimum* did not compete favourably with a known drug of choice for typhoid fever. Consequently, ethanolic leaf extract of *Ocimum gratissimum* which enhances erythropoiesis, may not be a potent immunostimulatory agent especially in an individual infected with *S. typhi*.

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