OPEN ACCESS
Saudi Journal of Medical and Pharmaceutical Sciences
Abbreviated Key Title: Saudi J Med Pharm Sci
ISSN 2413-4929 (Print) |ISSN 2413-4910 (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: https://saudijournals.com/journal/sjmps/home

Original Research Article

Immunomodulatory Effect of Aqueous Extract of Leaves of *Ocimum gratissimum* on Albino Wistar Rats

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| **Received:** 02.02.2019 | **Accepted:** 11.02.2019 | **Published:** 20.02.2019
DOI: 10.36348/sjmps.2019.v05i02.006

Abstract

Increased morbidity and mortality has been recorded from diseases such as HIV/AIDS, tuberculosis, hypersensitivity reactions, autoimmune disorders, and graft versus tissue rejections. Therefore, immunomodulation can be beneficial depending on the desired immune status. The aim of this study was to determine the effect of aqueous extract of leaves of *Ocimum gratissimum* on immune response in albino Wistar rats. Albino Wistar rats were administered the following extracts/drugs by oral administration for 21 days: Group 1 (40 mg/kg extract), Group 2 (80 mg/kg extract), Group 3 (60 mg/kg extract), Group 4 (Levamisole 0.5 mg/kg) positive control, Group 5 (no drugs, no extract) negative control. After 21 days, blood samples were collected for the estimation of plasma IgG level and absolute WBC count. There were statistically significant reductions in plasma IgG levels in the test groups compared to controls (p value 0.052). Multiple comparisons (Dunn's) of the group values further revealed statistically significant p values of 0.022 (group 2 vs group 1), 0.023 (group 2 vs group 4), and 0.011 (group 5 vs group 2). There were also statistically significant reductions in total WBC in the test groups compared to controls (p value 0.043). Multiple comparisons (Dunn's) of the group values further revealed statistically significant p values of 0.013 (group 2 vs group 5), and 0.010 (group 3 vs group 5). At the doses and concentrations used in this study, the aqueous leaf extract of *O. gratissimum* produced dose dependent inhibition of plasma IgG concentration and total WBC count in albino Wistar rats.

Keywords: Extract, IgG, Immunomodulation, Levamisole, *Ocimum gratissimum*, Rats.

INTRODUCTION

Immune response is the reaction of the cells and fluids of the body to the presence of foreign substances in the body. Thus immune response constitutes the major mechanism for the upkeep of the general well-being of an individual. The lymphocytes, macrophages, neutrophils, mast cells, eosinophils, basophils, and natural killer cells are the main cells involved in immune response. These cells identify and eliminate pathogens by engulfing and then killing microorganisms or by the activation of the adaptive immune system.

Humoral immune responses are often initiated by antigens in the presence of already formed antibodies. The antibodies include IgG, IgA, IgM, IgE, IgD. The IgG class which has a plasma concentration of 10-15 ng/ml with intracellular and extracellular distribution is the most abundant, constituting about 75% of immunoglobulins in the human.

At times the immune system fails to distinguish between self and non-self (foreign) antigens such that the immune response is directed against host tissues (autoimmunity). Also, the immune response can become exaggerated such that the response becomes injurious to host tissues (hypersensitivity reaction). Helminthic infestations are known to predispose to hypersensitivity reactions by modulating the Th1/Th2 response and dendritic cell functions [1, 2].

At other times the immune response can be defective (immunodeficiency). This can occur when one or more of the components of the immune system are inactive. The ability of the immune system to respond to pathogens is diminished in both extremes of life due to prematurity and decline in function of the organs respectively [3, 4]. Obesity, alcoholism, malnutrition, and drug use, sleep deprivation, and hypovitaminosis D are modifiable causes of immunodeficiency [5, 6].

Reduced plasma immunoglobulins are found in trauma, burns, nephritic syndrome, malignancy and drugs abuse [7]. On the other hand, IgG levels are increased in chronic infections such as HIV, chronic hepatitis and multiple sclerosis. In fact, increased IgG level is a diagnostic biomarker for autoimmune hepatitis [8]. The immune response can be manipulated to suppress unwanted responses as a result of hypersensitivity or autoimmunity, and to stimulate protective responses in cases of immunodeficiency which could lead to increased susceptibility to infections or cancer [9].

Justification/Rationale for the Study

Increased morbidity and mortality has been recorded from diseases such as HIV/AIDS, tuberculosis and even cancers as a result of immunosuppression. In 2017, the WHO estimated that 36.9 million people were living with HIV/AIDS and that 1 million people died of HIV-related illnesses worldwide [10]. The incidence and prevalence of pulmonary tuberculosis is also on the increase since HIV and tuberculosis are co-morbid infections that thrive on immuosuppression.

On the other hand excessive immunostimulation as exemplified by autoimmune diseases, hypersensitivity reactions, and graft versus tissue rejections negatively affects public health. Therefore, immunomodulation (the alteration of immune response to either increase or reduce it) can be beneficial depending on the desired immune status.

Consequently there is the need to look for more agents that will help prevent diseases by modulating immune responses.. So in this study, we intend to look at the effect of leaf extract of *Ocimum gratissimum* on immune response in Albino Wistar rats.

Ocimum gratissimum is a vegetable that is edible, so the chances of it being toxic are minimal. In fact, it has been demonstrated that its extract, when administered to albino rats for 28 days at a dose of 80 mg/kg did not result in any toxic effect to the liver [11]. This plant, also known as scent leaf (*nchuanwu* in Igbo) belongs to the family *Lamiaceaae* and is commonly used in the treatment of fever, diarrhoea, dysentery, pile, and convulsions [12]. It is also known to possess hepatoprotective properties [11]. Previous phytochemical studies on this plant revealed the presence of alkaloids, phytates, tannins, flavonoids, and oligosaccharides [13].

In general, the use of medicinal plants for therapeutic purposes will reduce the burden on available synthetic agents and also reduce the possibility of drug resistance to antimicrobials. In particular, this study will help to exploit *Ocimum gratissimum* for drug discovery and development. The extracts of this plant when refined and properly packaged may offer cheap and less toxic immunomodulatory alternative for the management of HIV, tunerculosis, hypersensitivity reactions, autoimmune disorders, and graft versus tissue rejections.

Aim of the Study

The aim of this study was to determine the effect of aqueous extract of leaves of *Ocimum gratissimum* on immune response in albino Wistar rats.

Objectives of the Study

The general objective was to determine the effect of the aqueous extract of leaves of O. gratissimum on immune response in albino Wistar rats. Specific Objectives included:

- To determine the effect of the aqueous extract of leaves of *O. gratissimum* on plasma immunoglobulin G (IgG) level in albino Wistar rats.
- To determine the effect of the aqueous extract of leaves of *O. gratissimum* on total WBC count in albino Wistar rats.
- To compare the effect of the extracts on plasma IgG level and total WBC count with that of levamisole.

MATERIALS AND METHODS

This study was conducted at the Laboratory, Department of Human Physiology, Faculty of Basic Medical Sciences, College of Medicine, Chukwuemeka Odumegwu Ojukwu University, Uli Campus. The procedures were in accordance with guidelines for the care and use of laboratory animals [14].

Calculation of Sample Size

Sample size of 25 rats at 95% power to detect a difference between means of 2.5 at a significant level (alpha) of 0.05 (two tailed) was chosen using the formula for the calculation of sample size for laboratory animals experiments [14]: N = 1+2C[s/d] 2.

Where, C = a constant (7.8) at 0.05 level of significance; s = 2.75 (standard deviation from a similar previous study) [15]; d = difference between means desired in present study.

Animal Source

Twenty five (25) male rats of 6-8 weeks old were obtained from the Animal House, Department of Human Physiology, Chukwuemeka Odumegwu Ojukwu University, Uli Campus, Nigeria. The animals were certified healthy by a veterinarian. Each group of 5 rats was housed in a metal cage measuring 60cm x 45cm x 30cm and was allowed free access to animal feeds which includes Growers and Top feeds, all made in Nigeria. Clean drinking water was also made available to the rats. Left over feeds and water were discarded and the cages were properly cleaned with chlorhexidine antiseptic solution every 12 hours. Artificial light was provided by fluorescent lamp

(Philips, Holland; 18 watts) and light-dark cycle of 12-12 hours was maintained. The animals were maintained in this arrangement throughout the duration of this study.

Drug Source

Levamisole (Dewormis, GlaxoSmithKline, India), diazepam injection (Valium, Roche, USA), and ketamine injection (Ketalar, Popular Pharmaceuticals, Bangladesh) were procured from Joez Pharmacy Limited, Awka, Anambra State.

Preparation of plant extracts

Three (3) kilograms of fresh leaves of O. gratissimum was collected, washed under running tap water, and air-dried at room temperature. Thereafter, the dried leaves were ground into fine powder and fifty grammes of the powder was extracted with 500 ml of distilled water using the Soxhlet method [11]. The filtrate was obtained by solvent evaporation and stored in refrigerator until use. Percentage yield of extract was determined using the formula:

$$\text{Percentage yield} = \frac{\text{Final weight of extract}}{\text{Weight of dry leaves}} \times 100$$

Experimental procedure

The rats were randomly divided into 5 groups of 5 rats each. Thereafter, we treated the animals with the following drugs by oral administration for 21 days [11] as follows:

Group 1: Each rat was given 40 mg/kg aqueous leaf extract of *O. gratissimum.*

Group 2: Each rat was given 80 mg/kg aqueous leaf extract of *O. gratissimum.*

Group 3: Each rat was given 60 mg/kg aqueous leaf extract of *O. gratissimum.*

Group 4: each rat was given Levamisole 0.5 mg/kg. This was used as the positive control

Group 5: Each rat was given normal feed (no drugs, no extract). This was used as the negative control.

A treatment chart was kept. After 21 days, blood samples were collected from each rat for the estimation of plasma IgG level and total WBC count.

Collection of Blood Samples

The rats were anesthetized individually using diazepam and ketamine. Thereafter, blood samples were collected using the method described by Hoff [16]. In brief, the skin over the jugular vein was cleaned with methylated spirit-soaked cotton wool and 2.0-2.5 ml of whole blood withdrawn through the jugular vein using a 25 guage hypodermic needle fitted unto a 2 ml syringe. About 1.5 ml of the withdrawn blood samples were transferred gently into EDTA bottle and allowed to clot naturally. Thereafter, the plasma was separated into specimen bottles using a micropipette and stored at 2-8°C until use within three days. The remaining 1 ml of withdrawn blood was quickly transferred into plain bottles containing anticoagulant (EDTA) for the WBC count.

Assays for plasma IgG were done using ELISA according to the manufacturer's instructions (Life Diagnostics, West Chester, USA). The optical densities were converted into mg/ml by means of digital software, Gen 5 (Diagnostic Automation, USA), attached to the microplate reader (Model No DAR 8000. Diagnostic Automation Inc., USA). The total WBC count was done using Haematology Autoanalyser (Erma Inc, PCE - 210)

Statistical Analysis

The data were tested for normality using D'Augostino and Pearson omnibus normality test. The mean values (± SEM) of plasma IgG concentrations and the total WBC were calculated and expressed in bar charts. The values were further analyzed for statistical significance and intergroup differences using analysis of variance (ANOVA) and Dunn's multiple comparisons respectively. There was no transformation of data. All analyses were done using SPSS version 21 and the results taken as statistically significant if the p-value < 0.05.

RESULTS

None of the animals died during the period of the experiment.

Yield of the Extract

Fifty grammes (50 g) of the powdered leaves yielded 19.8 grammes of extract giving a percentage yield of 39.6%.

Plasma IgG concentration

Mean values of plasma IgG concentration obtained (Table-1) fell within the normal values of 10-15 mg/ml. When subjected to analysis of variance (ANOVA) there were statistically significant results (p value 0.052) as shown in Table-2. Multiple comparisons (Dunn's) of the group values revealed statistically significant p values of 0.022 (group 2 vs group 1), 0.023 (group 2 vs group 4), and 0.011 (group 5 vs group 2) as shown in Table-3.

Total WBC count

The total WBC count (Table-1) also fell within the reference values of 6.6-12 (x 10^9/L). When the

values were subjected to ANOVA, there was also statistically significant result (p value 0.043) as shown in Table-2. Multiple comparisons (Dunn's) of the group values revealed statistically significant p values of 0.013 (group 2 vs group 5), and 0.010 (group 3 vs group 5) as shown in Table-4.

Bar chart representations of plasma IgG and total WBC counts of the test and control groups are shown in Figures 1 and 2 respectively.

Table-1: Mean (± SEM) plasma IgG (mg/dl) and total WBC count (x 10^9/L) of male albino Wistar rats after 21 day oral administration of extracts of *O. gratissimum* and levamisole to test and control groups of male albino Wistar rats

	Group 1				
	Sample 1	**Sample 2**	**Sample 3**	**Sample 4**	**Mean(± SEM)**
Plasma IgG (mg/dl)	1047.59	1139.01	1235.67	909.12	1082.85±138.97
WBC (x 10^9/L)	9.2	13.5	14.5	8.7	11.48±2.95
	Group 2				
	Sample 1	**Sample 2**	**Sample 3**	**Sample 4**	**Mean(± SEM)**
Plasma IgG (mg/dl)	901.28	893.45	864.71	1039.74	924.79±8.23
WBC (x 10^9/L)	12.6	10.0	7.2	8.5	9.55±2.32
	Group 3				
	Sample 1	**Sample 2**	**Sample 3**	**Sample 4**	**Mean(± SEM)**
Plasma IgG (mg/dl)	909.1	1000.56	987.49	1078.93	994.03±69.53
WBC (x 10^9/L)	8.9	9.7	7.3	11.4	9.33±1.71
	Group 4				
	Sample 1	**Sample 2**	**Sample 3**	**Sample 4**	**Mean(± SEM)**
Plasma IgG (mg/dl)	1102.44	1136.40	1063.25	1021.46	1080.89±49.63
WBC (x 10^9/L)	13.2	9.6	13.4	16.3	13.13±2.74
	Group 5				
	Sample 1	**Sample 2**	**Sample 3**	**Sample 4**	**Mean(± SEM)**
Plasma IgG (mg/dl)	1201.71	1024.07	1089.39	1102.44	1104.40±73.37
WBC (x 10^9/L)	18.4	16.0	12.3	12.3	14.75±2.99

Group 1 (40 mg/kg), Group 2 (80 mg/kg), Group 3 (60 mg/kg) of aqueous leaf extract of *O. gratissimum*, 0.5 mg/kg levamisole (Group 4), and normal feed (Group 5).

Table-2: Result of statistical significance test (ANOVA) on plasma IgG (mg/dL) and total WBC count (x10^9/L) obtained after 21 days of oral administration of extracts of *O. gratissimum* and levamisole to test and control groups of male albino Wistar rats respectively

ANOVA						
		Sum of Squares	Df	Mean Square	F	Sig.(P-value)
IgG (mg/dL)	Between Groups	92026.748	4	23006.687	3.018	.052
	Within Groups	114335.583	15	7622.372		
	Total	206362.330	19			
WBC(x10^9/L)	Between Groups	86.110	4	21.528	3.214	.043
	Within Groups	100.460	15	6.697		
	Total	186.570	19			

Table-3: Multiple comparisons of plasma IgG values obtained after 21 days of oral administration of extracts of *O. gratissimum* and levamisole to test and control groups of male albino Wistar rats respectively

Multiple Comparisons							
LSD							
Dependent Variable	(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
IgG (mg/dL)	Group 2	Group 3	-69.23000	61.73480	.280	-200.8146	62.3546
		Group 1	-158.05000*	61.73480	.022	-289.6346	-26.4654
		Group 5	-179.60500*	61.73480	.011	-311.1896	-48.0204
		Group 4	-156.09250*	61.73480	.023	-287.6771	-24.5079
	Group 3	Group 2	69.23000	61.73480	.280	-62.3546	200.8146
		Group 1	-88.82000	61.73480	.171	-220.4046	42.7646
		Group 5	-110.37500	61.73480	.094	-241.9596	21.2096
		Group 4	-86.86250	61.73480	.180	-218.4471	44.7221
	Group 1	Group 2	158.05000*	61.73480	.022	26.4654	289.6346
		Group 3	88.82000	61.73480	.171	-42.7646	220.4046
		Group 5	-21.55500	61.73480	.732	-153.1396	110.0296
		Group 4	1.95750	61.73480	.975	-129.6271	133.5421
	Group 5	Group 2	179.60500*	61.73480	.011	48.0204	311.1896
		Group 3	110.37500	61.73480	.094	-21.2096	241.9596
		Group 1	21.55500	61.73480	.732	-110.0296	153.1396
		Group 4	23.51250	61.73480	.709	-108.0721	155.0971
	Group 4	Group 2	ss156.09250*	61.73480	.023	24.5079	287.6771
		Group 3	86.86250	61.73480	.180	-44.7221	218.4471
		Group 1	-1.95750	61.73480	.975	-133.5421	129.6271
		Group 5	-23.51250	61.73480	.709	-155.0971	108.0721
*. The mean difference is significant at the 0.05 level.							

Table-4: Multiple comparisons of total WBC counts obtained after 21 days of oral administration of extracts of *O. gratissimum* and levamisole to test and control groups of male albino Wistar rats

Multiple Comparisons							
LSD							
Dependent Variable	(I) group	(j) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
	Group 2	Group 3	.25000	1.82994	.893	-3.6504	4.1504
		Group 1	-1.90000	1.82994	.316	-5.8004	2.0004
		Group 5	-5.17500*	1.82994	.013	-9.0754	-1.2746
		Group 4	-3.55000	1.82994	.071	-7.4504	.3504
	Group 3	Group 2	-.25000	1.82994	.893	-4.1504	3.6504
		Group 1	-2.15000	1.82994	.258	-6.0504	1.7504
		Group 5	-5.42500*	1.82994	.010	-9.3254	-1.5246
		Group 4	-3.80000	1.82994	.055	-7.7004	.1004
	Group 1	Group 2	1.90000	1.82994	.316	-2.0004	5.8004
		Group 3	2.15000	1.82994	.258	-1.7504	6.0504
		Group 5	-3.27500	1.82994	.094	-7.1754	.6254
		Group 4	-1.65000	1.82994	.381	-5.5504	2.2504
	Group 5	Group 2	5.17500*	1.82994	.013	1.2746	9.0754
		Group 3	5.42500*	1.82994	.010	1.5246	9.3254
		Group 1	3.27500	1.82994	.094	-.6254	7.1754
		Group 4	1.62500	1.82994	.389	-2.2754	5.5254
	Group 4	Group 2	3.55000	1.82994	.071	-.3504	7.4504
		Group 3	3.80000	1.82994	.055	-.1004	7.7004
		Group 1	1.65000	1.82994	.381	-2.2504	5.5504
		Group 5	-1.62500	1.82994	.389	-5.5254	2.2754
*The mean difference is significant at the 0.05 level.							

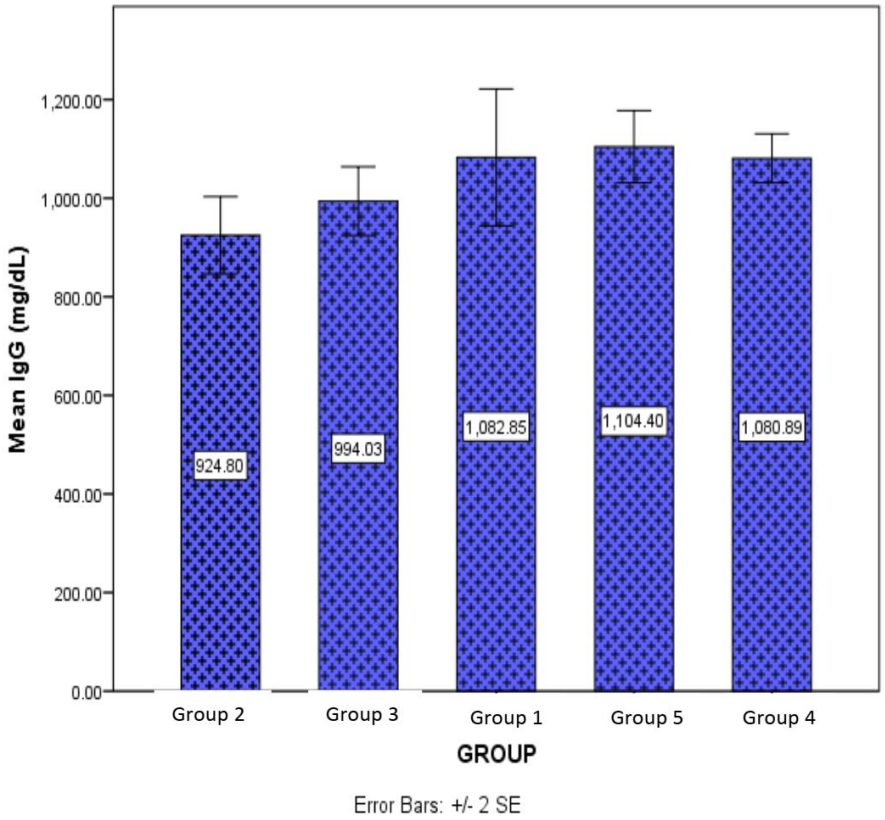

Fig-1: Bar chart comparing the mean plasma IgG for the test and control groups of male albino Wistar rats after 21 day oral administration of extracts of 40 mg/kg (Group 1), 80 mg/kg (Group 2), 60 mg/kg (Group 3) of aqueous leaf extract of *O. gratissimum;* 0.5 mg/kg levamisole (Group 4); and normal feed (Group 5)

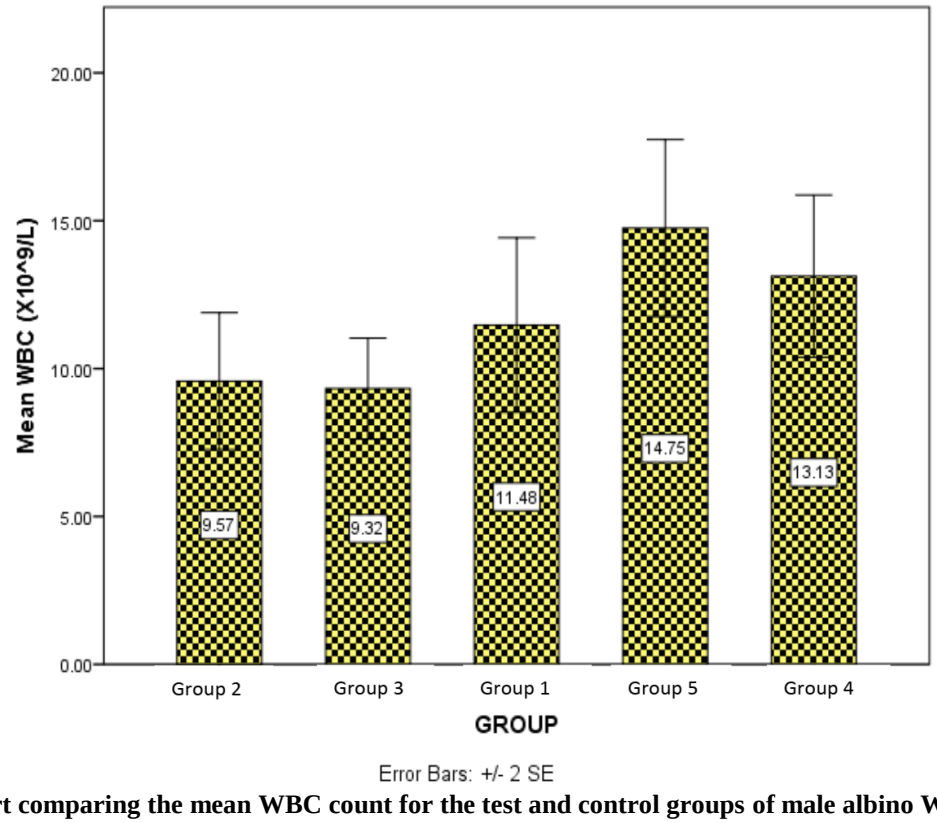

Fig-2: Bar chart comparing the mean WBC count for the test and control groups of male albino Wistar rats after 21 day oral administration of extracts of 40 mg/kg (Group 1), 80 mg/kg (Group 2), 60 mg/kg (Group 3) of aqueous leaf extract of *O. gratissimum;* 0.5 mg/kg levamisole (Group 4); and normal feed (Group 5)

DISCUSSION

Mean values of plasma IgG concentration obtained (Table I) fell within the normal values of 10-15 mg/ml (1000-1500 mg/dl). Group 1 rats (fed with extract 40 mg/kg) had mean plasma IgG of 1082.85±138.97 which is similar to those of group 4, levamisole (1080.89±49.63) and group 5, negative control (1104.40±73.37). However, group 2 rats (fed with extract 80 mg/kg) had low plasma IgG compared to groups 4 and 5 as shown by multiple comparison p-values of 0.23 and 0.11 respectively. Therefore, the extract can be said to have produced a statistically significant lower plasma IgG level compared to the positive and negative controls. This implies that the extract is immunosuppressive in this study. There are no published results on the effect of this extract on plasma IgG in rats. However, there are reports of the anti-inflammatory effects of the extract [17]. Since this extract has anti-inflammatory properties, it will most likely possess immunosuppressive properties because some anti-inflammatory cytokines also have immunosuppressive actions. Such cytokines include IL-1, IL-4, IL-10, IL-13, and TNF-β. In particular, the primary function of IL-10 is to limit and possibly terminate inflammatory response and to regulate the differentiation and proliferation of many immune cells [18].

The immunosuppressive action of the extract was dose dependent since group 2 rats (80 mg/kg, group 3 rats (60 mg/kg), and group 1 rats (40 mg/kg) had plasma IgG concentrations (mg/dl) of 924.79±8.23, 994.03±69.53, 1082.85±138.97 respectively.

Plasma IgG levels are considered indicative of an individual's immune status to a particular pathogen. Because of its relative abundance and antigen specificity IgG is the principal antibody used in immunological research and clinical diagnosis [19].

There are four IgG subclasses (IgG1, IgG2, IgG3, IgG4), IgG1 being the most abundant. It would have been ideal to measure the concentration of the different subclasses to find out the effect of the extract on the subclasses since IgG antibody response to certain antigens occur more in one type of IgG subclass than others [20]. The implication is that some persons with normal plasma levels of total IgG may become susceptible to certain infections because of the deficiency in one IgG subclass. Since IgG1 comprises 60% of the total IgG level, deficiency of IgG1 usually drops the total IgG level below the normal range, resulting in hypogammaglobulinemia. However, assessing IgG subclasses adds cost and is not always reliable so that all abnormal values need to be repeated at least once in a separate blood sample [21].

The use of medicinal plants for therapeutic purposes will reduce the burden on available synthetic agents and also reduce the possibility of drug resistance to antimicrobials. In particular, this study will help to exploit Ocimum gratissimum for drug discovery and development. The extracts of this plant when refined and properly packaged may offer cheap and less toxic immunomodulatory alternative for the treatment of hypersensitivity reactions, autoimmune disorders, and graft versus tissue rejections.

For the WBC, group 1 rats (extract 40 mg/kg) had total WBC count of 11.48± 2.95 $x10^9$/L which was close to 13.13±2.74 $x10^9$/L (that of the positive control) whereas group 2 rats (80 mg/kg) and group 3 rats (60 mg/kg) had total WBC counts of 9.55±2.32 $x10^9$/L and 9.33±1.71 $x10^9$/L respectively. However, when compared to group 5 using Dunn's multiple comparison test, group 2 and group 3 produced statistically significant p-values of 0.013 (group 2 vs group 5) and 0.010 (group 3 vs group 5) respectively.

It can be seen that the effect of the abstract on total WBC is inhibitory and dose-dependent. This inhibitory action on WBC is in agreement with the inhibitory effect on plasma IgG already established above. Since WBC and IgG collaborate to mediate humoral immune response, the extract can be said to be immunosuppressive.

The reduction in total WBC count agrees with the results obtained in previous studies [22, 23]. The reduction in total WBC count and plasma IgG level could be due to the presence of saponins in the plant extract. Saponins are known to reduce total WBC count and to generally decrease the function of the haemopoietic system [24]. The oncurrent inhibition of WBC count and plasma IgG level is not surprising since B lymphocytes (component of WBC) are responsible for production of plasma cells which in turn produce antibodies (immunoglobulins) including IgG.

CONCLUSION

At the doses and concentrations used in this study, this extract of leaves of *O. gratissimum* produced dose dependent inhibition of plasma Igg concentration and total WBC count in albino Wistar rats.

The measurement of the effect of the extract on specific IgG subclasses would have been more ideal since the immunomodulatory effect of the subclasses differ depending on the individual and disease types.

Further studies on this extract should involve clinical trials; more so since this extract has been found to be non-toxic to humans in previous studies.

REFERENCES

1. Carvalho, L., Sun, J., Kane, C., Marshall, F., Krawczyk, C., & Pearce, E. J. (2009). Review series on helminths, immune modulation and the hygiene hypothesis: mechanisms underlying

helminth modulation of dendritic cell function. *Immunology*, *126*(1), 28-34.
2. Fujiwara, R. T., Cançado, G. G., Freitas, P. A., Santiago, H. C., Massara, C. L., dos Santos Carvalho, O., ... & Bethony, J. (2009). Necator americanus infection: a possible cause of altered dendritic cell differentiation and eosinophil profile in chronically infected individuals. *PLoS Neglected Tropical Diseases*, *3*(3), e399.
3. Chandra, R. K. (1997). Nutrition and the immune system: an introduction. *The American journal of clinical nutrition*, *66*(2), 460S-463S.
4. Aw, D., Silva, A. B., & Palmer, D. B. (2007). Immunosenescence: emerging challenges for an ageing population. *Immunology*, *120*(4), 435-446.
5. Krueger, J. M., & Majde, J. A. (2003). Humoral links between sleep and the immune system: research issues. *Annals of the New York Academy of Sciences*, *992*(1), 9-20.
6. Bryant, P. A., Trinder, J., & Curtis, N. (2004). Sick and tired: does sleep have a vital role in the immune system?. *Nature Reviews Immunology*, *4*(6), 457-467.
7. Agarwal, S., & Cunningham-Rundles, C. (2007). Assessment and clinical interpretation of reduced IgG values. *Annals of Allergy, Asthma & Immunology*, *99*(3), 281-283.
8. Czaja, A. J. (1996). Diagnosis and therapy of autoimmune liver disease. *Medical Clinics of North America*, *80*(5), 973-994.
9. Miller, J. F. (2002). The discovery of thymus function and of thymus-derived lymphocytes. *Immunological reviews*, *185*(1), 7-14.
10. *Avert. (2018). Global HIV and AIDS statistics, Available at:* https://www.avert.org/global-hiv-and-aids-statistics. *(Accessed 21 august 2018).*
11. Ughachukwu, P., Okonkwo, P., Nwozor, C., Okafor, J., & Nwafia, I. (2016). Effect of Aqueous Leaf Extract of Ocimum gratissimum on Antiretroviral Drug-Induced Hepatotoxicity in Rats. *European Journal of Medicinal Plants*, *13*(1), 1-11.
12. Prabhu, K. S., Lobo, R., Shirwaikar, A. A., & Shirwaikar, A. (2009). Ocimum gratissimum: A review of its chemical, pharmacological and ethnomedicinal properties. *The Open Complementary Medicine Journal*, *1*(1-15).
13. de Vasconcelos Silva, M. G., de Abreu Matos, F. J., & Roberto, P. (2004). Composition of essential oils from three Ocimum species obtained by steam and microwave distillation and supercritical CO2 extraction. *Arkivoc*, *6*(2004), 66-71.
14. National Research Council. (2003). *Guidelines for the care and use of mammals in neuroscience and behavioral research*. National Academies Press.
15. Youm, J. W., Won, Y. S., Jeon, J. H., Moon, K. B., Kim, H. C., Shin, K. S., ... & Kim, H. S. (2010). Antibody responses in mice stimulated by various doses of the potato-derived major surface antigen of hepatitis B virus. *Clin. Vaccine Immunol.*, *17*(12), 2029-2032.
16. Hoff, J. (2000). Methods of blood collection in the mouse, *Lab Animal*, 29(10), 47-53.
17. Olamilosoye, K. P., Akomolafe, R. O., Akinsomisoye, O. S., Adefisayo, M. A., & Alabi, Q. K. (2018). The aqueous extract of Ocimum gratissimum leaves ameliorates acetic acid-induced colitis via improving antioxidant status and hematological parameters in male Wistar rats. *Egyptian Journal of Basic and Applied Sciences*, *5*(3), 220-227.
18. Shao, Y., Cheng, Z., Li, X., Chernaya, V., Wang, H., & Yang, X. F. (2014). Immunosuppressive/anti-inflammatory cytokines directly and indirectly inhibit endothelial dysfunction-a novel mechanism for maintaining vascular function. *Journal of hematology & oncology*, *7*(1), 80.
19. Thermo Fisher Scientific, USA. (2019). Immunoglobulin IgG class. Available at: http://www.thermofisher.com/us/en/home/life-science/antibodies (Accessed 21 January 2019).
20. Laboratory Corporation of America, LabCorp. (2019). Immunoglobulin G, Subclasses (1-4). Available at: https://www.labcorp.com/test-menu/29431/immunoglobulin-g-subclasses-1-4 (Accessed 21 January 2019).
21. Immune Deficiency Foundation. (2019). IgG Subclass Deficiency. Available at: https://primaryimmune.org/about-primary-immunodeficiencies/specific-disease-types/igg-subclass-deficiency/ (Accessed 15 December, 2018).
22. Ephraim, K. D., Salami, H. A., & Osewa, T. S. (2000). Effect of aqueous leaf extract of Ocimum gratissimum on heamatological and biochemical parameters in rabbits. *African Journal of Biomedical Research*, *3*, 175-179.
23. Jimoh, O. R., Olaore, J., Olayaki, L. A., Olawepo, A., & Biliaminu, S. A. (2008). Effects of aqueous extract of Ocimum gratissimum on haematological parameters of Wistar rats. *Biokemistri*, *20*(1) 33-37.
24. Saeed, M. A., & Sabir, A. W. (2003). Effects of Fagonia cretica L. constituents on various haematological parameters in rabbits. *Journal of ethnopharmacology*, *85*(2-3), 195-200.