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Assessment of *Ocimum gratissimum* leaves on Hematological parameters and Cell-mediated immunity of Rabbits

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Abstract

The effects of the plant *Ocimum gratissimum* was assessed on some hematological parameters including white blood cell (WBC) indices, packed cell volume (PCV), hemoglobin (Hb) and platelet count and cell-mediated aspect of immunity (CD_4 count and secretion of 1nterleukin-2) on rabbits after a repeated administration of the plant additive at different doses for a period of eight (8) weeks were determined. The results showed a significant increase ($P < 0.05$) in all the hematological parameters studied in a dose dependent manner. The results obtained also showed that *O. gratissimum* - supplemented diet caused a significant increase ($P < 0.05$) in the CD_4 count and secretion of interleukin-2 which is a hematopoietin; hence enhancing immune response.

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Introduction

Ocimum gratissimum (commonly known as scent leaf) is a herbaceous plant which belongs to the Labiatae family. The plant is indigenous to tropical areas especially India and it is also in West Africa. In Nigeria, it is found in the Savannah and coastal areas. In the southern part of Nigeria, the plant is called "effinrin-nla" by the Yoruba speaking tribe. It is called "Ahuji" by the Igbos, while in the Northern part of Nigeria, the Hausas call it "Daidoya" (Effraim *et al.*, 2003).

Phytochemical evaluation of this plant has shown that it is rich in alkaloid, tannis, phytates, flavonoids and oligosaccharides. The volatile aromatic oil from the leaves consists mainly of thymol (32-65%) and eugenol; it also contains xanthones, terpenes and lactones (Ezekwesili *et al.*, 2004). In folk medicine, *O. gratissimum* is extensively used throughout West Africa as a febrifuge, anti-malarial and anti-convulsant; the crushed leaf juice is used in the treatment of convulsion, stomach pain and catarrh while oil from the leaves have been found to possess antiseptics, antibacterial and antifungal activities (Ezekwesili *et al.*, 2004; Iwu,1986). In the coastal area of Nigeria, the plant is used in the treatment of epilepsy, high fever and diarrhea, (Osifo, 1992) while in the savannah areas, decoctions of the leaves are used to treat mental illness (Oliver, 1980; Sofowara, 1993). People of different cultures across the globe like in India, Kenya and some sub Saharan African communities' use this plant for various purposes such as treatments of sunstroke, headache, as a diaphoretic, antipyretic for its anti-inflammatory activity, abdominal pains, sore eyes, ear infections, coughs, barrenness, fever, convulsions, tooth gargle, regulation of menstruation and as a cure for prolapse of the rectum (Ta'nia *et al.*, 2006; Matasyoh *et al.*, 2007).

Hematological parameters are those parameters that are related to the blood and blood forming organs (Waugh *et al.*, 2001; Bamishaiye *et al.*, 2009). They are good indicators of the physiological status of animals so that they are often used to determine various status of the body and to determine stresses due to environmental, nutritional and/or pathological factors (Khan and Zafar, 2005). The cell-mediated component of immunity is mediated by T lymphocytes, the predominant cell types being helper T cells (Th) and cytotoxic T cells. T lymphocytes act through secretion of cytokines (such as interleukins) to elaborate and prime the immune response. T lymphocytes are characterized by expression of CD_4 proteins and are activated when MHC type II molecules, expressed on professional antigen-presenting cells (dendritic cells, macrophages, and B cells), activate the specific T cell receptor (Nagarathna *et al.*, 2013).

Relatively, little research work has been on the effect of *O. gratissimum* on any aspect of the immune system. Hence, the aim of this study therefore is to assess the effects of this plant additive on some hematological parameters and its ability to stimulate cell-mediated immunity in rabbits.

Materials and methods

Collection and processing of leaves

The leaves of *O. gratissimum* were purchased from New Benin Market, Benin City, Edo State, Nigeria. The leaves were collected and picked thoroughly to remove debris and rotten leaves and washed thoroughly with water and then air-dried under a shade for ten (10) days (Ijeh *et al.*, 2005). When the leaves were well-dried, they were ground into coarse powder using a modern laboratory electric milling machine (Chris Norris, England).

Formulation of experimental diets

Two experimental diets namely Control Diet (CD) and *O. gratissimum* -supplemented diet (OGSD) were formulated. The diets were formulated from commercially available feed grade feedstuffs (TopFeed grower's mash) which included maize, corn flour, fish meal, groundnut meal, bone meal and vitamin premix. The control diet (CD) was formulated without the inclusion of *O. gratissimum* powder while the *O. gratissimum*-supplemented diet (OGSD) was incorporated with 5%, 10% and 20% of *O. gratissimum* powder.

Experimental Design

This involved a random distribution of twenty-four adult Chinchilla rabbits into two experimental groups; Control Group (CG) and *O. gratissimum* group (OGSG) consisting of four animals each. The animals in CG were fed control diet (CD) while those in OGSG were fed *O. gratissimum*-supplemented diet (OGSD) in 5%, 10%, 20% and given water *ad libitum* for nine weeks. Blood samples (3 ml) were collected from the marginal ear vein of the rabbits in the two groups into EDTA coated tubes for analysis on a fortnightly basis.

Hematological analysis

The hematological assessment of each sample was done in accordance with the directions on the Sysmex KX-21N Automated

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Hematology Analyser (Dacie and Lewis, 1984). The following parameters were assessed white blood cell count (WBC), packed cell volume (PCV), hemoglobin (Hb), neutrophils, lymphocyte and platelet counts.

T-lymphocyte assessment (CD_4^+ count)

Cluster of differentiation (CD_4^+) lymphocytes frequency was determined by the Flow cytometry method. The name of the kit used for this work is the CD_4 easy count kit (manufactured by Partec Cooperation, Germany).

Determination of Cytokine Secretion

The concentrations of interleukin 2 (IL-2) in the rabbits' sera were determined by specific quantitative sandwich ELISA kits according to the instruction of the manufacturer.

Statistical analysis

The data obtained were presented as mean ± SEM (standard error of mean) by using the SPSS software package. The mean values between test groups and control were analyzed by using the Students' T-test for statistical difference at 95% confidence limits. Duncan Multiple Range Test was used to locate the source of the significant difference in some cases.

Results

The results of the screening for the effects of the plant additive on different hematological parameters such as white blood cell count (WBC), packed cell volume (PCV), hemoglobin (Hb), neutrophils, lymphocytes and platelet count on the rabbits after a repeated administration of the leaves of *O. gratissimum* for a period of eight (8) weeks showed a significant increase ($P < 0.05$) in all the parameters studied in a dose dependent manner (Tables 1).

Table 1: Hematological parameters of rabbits fed with *O. gratissimum*

Parameter	Control	5%	10%	20%
WBCX10^3 (µl)	3.70 ± 0.25	6.60 ± 0.11[a]	6.90 ± 0.60 [a]	6.90 ± 0.30 [a]
PCV (%)	29 ± 0.71	38 ± 1.83[b]	42 ± 1.85 [a]	43 ± 1.09 [a]
Hemoglobin (g/dl)	10.50 ± 0.40	13.20 ± 0.45 [b]	14.00 ± 0.12 [a]	14.60 ±0.40 [a]
Neutrophils (%)	30.70 ± 0.95	50.20 ± 0.90 [a]	50.70 ± 0.79 [a]	51.40± 1.27 [a]
Lymphocytes (%)	48.80 ± 1.31	51.90 ± 1.35 [b]	62.10 ± 0.96 [a]	65.50 ±1.69 [a]
Platelets x10^4/ml	14.80 ± 0.22	29.60 ± 1.33 [b]	32.20±1.07 [a]	34.20±1.46 [a]

Values = Mean ± S.E.M. Using Duncan Multiple Range Test. Alphabets of same letters are significant at $P < 0.05$ within rows

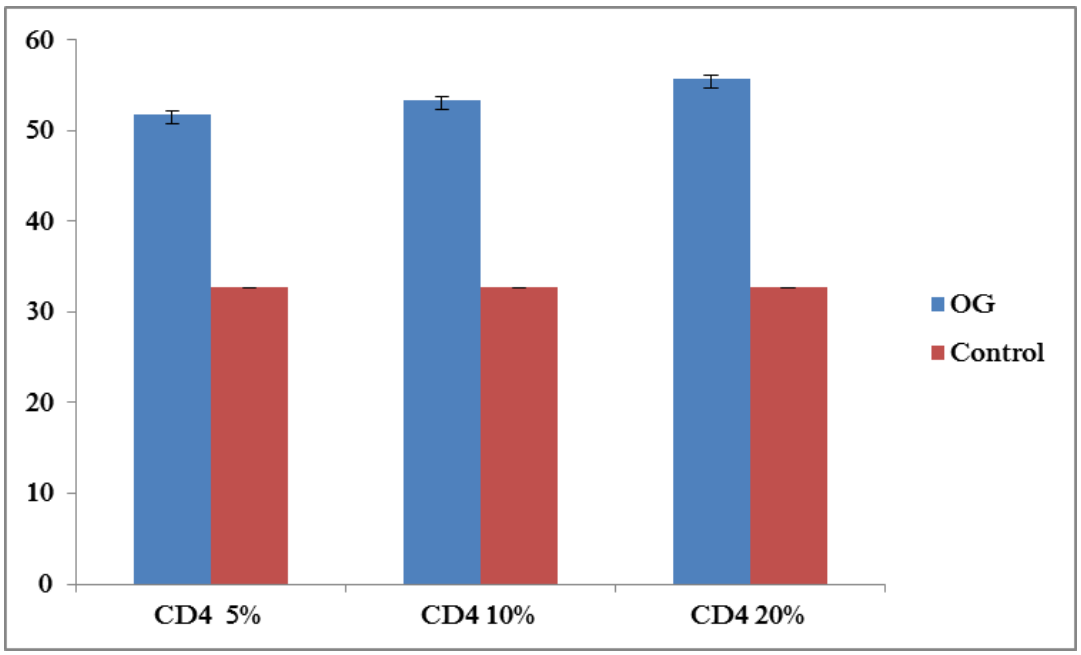

Figure 1: Effects of O. gratissimum additive on T lymphocyte proliferation (CD_4 count) of rabbits at 5%, 10% and 20%.

The effects of the plant additive on the proliferation of T lymphocytes (CD_4 count) revealed that there was a consistent significant increase in the CD_4 count when compared with the control group. The increase caused by *O. gratissimum* is shown in Figure 1 and was also observed in a dose dependent manner (among the different doses).

The effects of the plant additives in stimulating cytokine secretion specifically interleukins-2 showed that there was a stable increase in IL-2 concentration in the treated groups and in all the doses in comparison to the control group (Figure 2).

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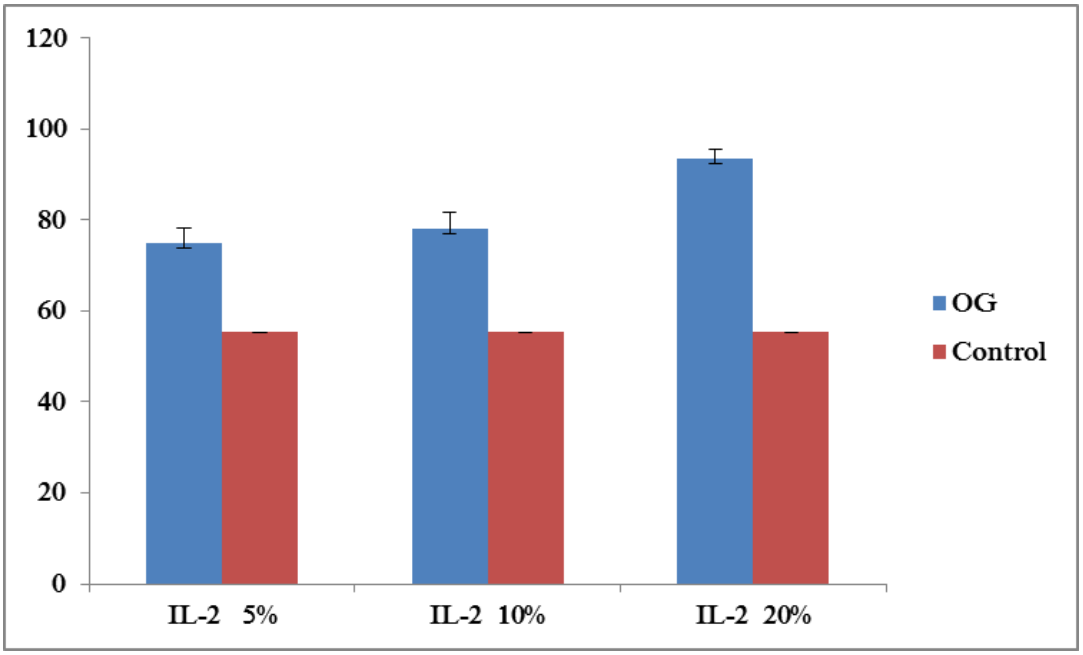

Figure 2: Effects of *O. gratissimum* (OG) additive on stimulation of Interleukins-2 production by rabbits at 5%, 10% and 20%.

Discussion

The major functions of the white blood cell and its differentials are to fight infections, defend the body by phagocytosis against invasion by foreign organisms and also to produce or at least transport and distribute antibodies in immune response. They also function in defending the immune system against antigens and invading microorganisms as well as viral-infected cells.

The neutrophils count was also correspondingly higher in groups treated with the different plant additives. Neutrophils are important phagocytic cells involved in innate surveillance and protection against a broad spectrum of pathogens and invaders. The platelets primarily function in secreting procoagulants to promote blood clotting. They also secrete chemicals that attract neutrophils and monocytes to sites of inflammation. Hemoglobin has the physiological function of transporting oxygen to tissues of the animal for oxidation of ingested food so as to release energy for the other body functions as well as transport carbon dioxide out of the body of animals (Ugwuene, 2011; Soetan *et al.*, 2013; Isaac *et al.*, 2013).

The effects of the plant additive on the proliferation of T lymphocytes (CD_4 count) revealed that there was a consistent significant increase in the CD_4 count when compared with the control group. It can be seen that in this study *O. gratissimum* has the potential to assist the immune system in T lymphocytes proliferation in a dose dependent manner. T cell lymphocytes constitute more than 60% of circulating lymphocytes and play a central role in both humoral and cell-mediated immunity (Jayapal, 2007). T cell can be further broadly classified as T helper lymphocytes (CD_4+), cytotoxic T lymphocytes (CD_8+) and regulatory T lymphocytes(T reg). Identification and enumeration of CD_4 T lymphocytes is important in many immunological experiments that involve T cell characterization or study of T cell function (Delves and Roitt, 1998). Whereby, several studies have shown that T lymphocytes (T cells) play critical roles in the regulation of immune responses, and are responsible for mediating many of the effectors' mechanisms of the immune system (Boyman and Sprent, 2012).

The T cells are activated and regulated by complex pathways involving a number of signal transduction molecules, including receptors for antigens and cytokines, kinases and transcription factors. When foreign antigens enter the body, they are recognized by the innate immune system, which in turn responds with the expression of surface co-stimulatory molecules and the release of cytokines. These expressed molecules inform the adaptive immune system about the type and strength of the offending pathogen.

Cytokines are proteins, essential mediators of cell-to-cell signals in physiological and pathological processes such as in the activation of immune responses, hematopoiesis, inflammation and homeostasis. Under normal conditions, these cytokines act as crucial signals in the development of appropriate defenses. The results in this study whereby the plant additive used was *O. gratissimum* enhanced IL-2 stimulation significantly in a dose - dependent manner, as revealed in Figures 2. Interleukin 2 (IL-2) is a hematopoietin which is produced by the Helper T cells (Th 1 cells). They are of biological importance as they help to activate T and B cells as well as natural killer (NK) cells. They function in cell growth, proliferation and synthesis of antibodies. Interleukin-2 (IL-2) signals influence various lymphocyte subsets during differentiation, immune responses and homeostasis. Boyman and Sprent, (2012) reported that the stimulation with IL-2 is crucial for the maintenance of regulatory T (T Reg) cells and for the differentiation of CD_4(+) T cells into defined effector T cell subsets following antigen-mediated activation.

Conclusion

This study has revealed that *O. gratissimum* has the ability to enhance some hematological indices and also stimulate cells of the immune system for proper functioning. These attributes could be traced to the qualitative phytochemical analysis of *O. gratissimum* which showed that it contains natural physiologically active substances such as terpenoids, flavonoids etc., which have also been reported in several research studies. The consumption of this plant additive for its health benefits should therefore be recommended.

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