

HEMATOLOGICAL AND IMMUNOMODULATORY ACTIVITY OF *Ocimum basilicum*

*¹ ILEV BARE LOVETH; ² EVBUOMWAN LUCKY

^{1,2}Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria.

*Corresponding author: evbuomwanlucky1@gmail.com

Abstract: This study was carried out to explore the immunomodulatory potential of aqueous leaf extract of *Ocimum basilicum*. Sixty four (64) Swiss albino rats (*Rattus norvegicus*) weighing between 120-180gm were used. They were divided into eight (8) groups of four (4) rats each. Group A, B and C comprised of immunosuppressed albino rats while D, E and F comprised of normal rats. Groups G and H were the negative and positive controls respectively. Aqueous extract of 200mg/kg, 400mg/kg and 600mg/kg were the concentrations of leaf extract used while 100mg/kg cyclophosphamide served as the immunosuppressant. Rats in group G (negative control) received feed and water only while Group H (positive control) received the immunosuppressant dose. Blood samples were withdrawn for determination of haematological parameters, humoral antibody response, CD4 count and phagocytic index. Phytochemical analysis of leaf extract was carried out using appropriate method. Results showed that hemoglobin (HGB) was highest in Group A (13.90 ± 0.74) and lowest in Group F (11.8 ± 1.18). RBC ranged from 4.03 ± 0.14 (Group H) to 6.63 ± 0.72 (Group C). CD₄ cells counts increased in Group C (13.5 ± 0.95) and, Group F (10.0 ± 0.70) treatment groups when compared with Group G (0.65 ± 0.28) and Group H (3.00 ± 0.57) control groups. Similar trend was observed in the humoral antibody response of the rats in Group C (7.11 ± 0.30) and Group F (11.21 ± 0.23) when compared to the positive (3.95 ± 0.31) and negative (2.12 ± 0.22) controls.

Keywords: Antibody, Antigen, Hemagglutination, Blood, Lymphocytes, Granulocytes, Immunosuppressant, phytochemicals.

1. INTRODUCTION

Medicinal plants are rich in secondary metabolites and essential oils of therapeutic importance. Screening of plants have revealed many compounds including flavonoids, alkaloids, saponins, terpenoids, monoterpenoids (linalool), glycoproteins, polysaccharides, tannins, essential fatty acids, phenolic compounds and vitamins having pronounced antioxidant, antineoplastic, antiulcer, anti-inflammatory and immunostimulating potentials (Wagner, 1990). The important advantages claimed for therapeutic uses of medicinal plants in various ailments are their safety besides being economical, effective and easily available. Because of these advantages medicinal plants have been widely used by traditional medical practitioners in their day to day practice. Among the plants known for their medicinal value, is the plants of genus *Ocimum* which are rich in phenolic compounds. *Ocimum basilicum* Linn. (Lamiaceae) is commonly known as Sweet basil. The word basil comes from the Greek (basileus), meaning "King", of the royal fragrance of this herb (Neelam *et al.*, 2010). *Ocimum basilicum* has been used for thousands of years as a medicinal herb. It acts principally on the digestive and nervous systems (Trease and Evans 1983; Kivilompolo and Hyötyläinen 2007). According to Ayurveda, *Ocimum basilicum* is used as an, anthelmintic and antipyretic herb. Its usefulness in diseases of the heart and blood has also been reported. Some studies have also reported its antioxidant, radical scavenging, anti-inflammatory and antiulcer activities

(Roshan and Savitri, 2013). In a study, aqueous and ethanolic extracts of leaves of *Ocimum basilicum* (OB) was administered orally at the dose of 400mg/kg/day in mice, and findings showed a significant increase in the production of circulating antibody titre in response to sheep red blood cells. A significant increase in both primary and secondary haemagglutination antibody (HA) titre was observed while compared to control group, whereas, in cyclophosphamide treated group showed significant increase in HA titre. OB significantly potentiated the delayed type hypersensitivity reaction by facilitating the footpad thickness response to sheep red blood cells in synthesized mice. Also OB evoked a significant increase in percentage neutrophil adhesion to Nylon fibres and phagocytic activity. The study demonstrates that OB triggers both specific and non-specific responses to a greater extent. From the results obtained and phytochemical studies, the immunostimulant effect of OB could be attributed to the flavonoid content (Neelam and Nilofer, 2010). The cytoprotective effects of rosmarinic acid against aflatoxin, mycotoxin and ochratoxin induced cytotoxicity and carcinogenicity was investigated in hepatoma-derived cell line (HepG2) of human. Rosmarinic acid dose dependently inhibited DNA and protein synthesis. Apoptosis cell death was prevented by reduction of DNA fragmentation and inhibition of caspase-3 activation (Renzulli *et al.*, 2004).

2. MATERIALS AND METHODS

Plant Material

Fresh *Ocimum basilicum* (curry leaf) leaves were purchased from new Benin Market, Benin City. Identification and authentication was done at the Herbarium of the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin where a voucher specimen number (UBH₀₃₃₃) has been preserved for future identification.

Extraction

The fresh leaves were separated from the stems and dried under shade at room temperature. The air-dried leaves were homogenized using a blender to obtain about 500g coarsely powdered leaf which was successively subjected to soxhlet extraction with water. The aqueous extract was evaporated under reduced pressure at low temperature (30°C) to dryness to yield a yellowish-brown extracts of *Ocimum basilicum*. The extract was stored in an airtight container in refrigerator for further experimental studies.

Phytochemical Screening

The aqueous extracts of *Ocimum basilicum* were subjected to phytochemical screening for the detection of various plants constituents following the method described by Trease and Evans (1993) and Kokate *et al.* (2001).

Animals

Inbred swiss albino rats (*Rattus norvegicus*) weighing between 120-180mg was housed in groups of 4 at the Department of Microbiology Animal House. All mice were feed with pelleted feed and water. Ethical approval for the experimental protocol was obtained from the University of Benin Ethics Committee and care of animals was taken as per guidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA).

Drugs and Chemicals

The drug and chemicals were of analytical grade. Cyclophosphamide (Biochem pharmaceutical, Mumbai) and Colloidal carbon (Indian ink, camel India Pvt. Ltd.) were used in the study.

Experimental Procedure

Antigenic Material: Preparation of Sheep RBCs (SRBCs)

Sheep blood was collected in sterile Alsevere's solution in 1:1 proportion of Alsevere's solution (freshly prepared). Blood was kept in the refrigerator and processed for the preparation of SRBCs batch by centrifuging at 2000 rpm for 10 minutes and washing with physiological saline 4-5 times and then suspending into buffered saline for further use (Gokhale *et al.*, 2003).

Carbon Ink Suspension

Indian ink was diluted eight times with saline and used for carbon clearance test in a dose of 10 µl/mg bw of mice (Dash *et al.*, 2006).

Haemagglutination Antibody (Ha) Titer

The animals were divided into eight groups consisting of four animals each. Immunosuppressed animals in group A, B and C were given extract (200 mg/kg), (400 mg/kg) and (600mg/kg) plus cyclophosphamide (100 mg/kg/p.o.) on the 9th day as a single dose respectively. Normal rats in treatment group D, E and F were given extract (200 mg/kg), (400 mg/kg) and (600mg/kg) daily for 14 days respectively. Rats in group G (positive control) received vehicle only for 14 days and Group H (negative control) received cyclophosphamide 100 mg/kg on the 9th day as a single dose. On the 7th day of the study, animals from all the groups (i.e. group I to VIII) were immunized with SRBCs in normal saline (0.1ml of 20% SRBCs) intraperitoneally. Blood was withdrawn on 14th day from retro-orbital plexus under mild anaesthesia from all antigenically sensitised mice. Blood was centrifuged to obtain serum, normal saline was used as a diluent and SRBCs count was adjusted to (0.1% of SRBCs). Each well of a microtitre plate was filled initially with 20 µl of saline and 20µl of serum was mixed in the first well of micro titer plate. Subsequently the 20 µl diluted serum was removed from first well and added to the next well to get twofold dilutions of the antibodies present in the serum. Further twofold dilutions of this diluted serum were similarly carried out till the last well of the second row (24th well), so that the antibody concentration of any of the dilutions is half of the previous dilution. 20µl SRBCs (0.1% of SRBCs) were added to each of these dilutions and the plates were incubated at 37°C for one hour and then observed for haemagglutination. The highest dilution giving haemagglutination was taken as the antibody titre (Joharapurkar *et al.*, 2004)

Carbon Clearance Test

The treatment was exactly the same as described above for HA titer. On 14th day, 3hrs after the last dose all the animals of each group were given colloidal carbon intravenously in a volume of 1ml/100g. Blood samples were then withdrawn (25 µl) from retroorbital plexus at 0 and 15 minutes after injection of colloidal carbon ink and lysed in sodium carbonate solution (3 ml). The optical density was measured spectrophotometrically at 650 nm (Gayathri *et al.*, 2005). The phagocytic index (K) was calculated using the formula:

$$K = (\ln OD1 - \ln OD2)/(t2 - t1)$$

Where, OD1 and OD2 are the optical densities at time t1 and t2 respectively.

Haematological Analysis

The treatment was exactly the same as described above for HA titer. On the 14th day, animals were sacrificed under mild anaesthesia and blood was withdrawn from the cardiac aorta into EDTA bottles for determination of haematological parameters following the method described by Ola-Davies *et al.* (2014).

CD₄ Cells Count

The experimental procedure was same as mentioned in the HA titre. The CD4 lymphocyte count was estimated by flow cytometry (Center for Disease Control and Prevention, 1997) using the flow automated cell counter (Partec, Germany). Ten microlitres (10µl) of CD4 PE antibody (Partec, Germany) was mixed with 50 ml of EDTA anticoagulated whole blood in test tubes. The mixture was incubated in the dark chamber for 15mins at room temperature (22-28°C). During incubation, the content of the tube was mixed every 5mins. Eight hundred microlitres (800µl) of buffer solution was added, mixed and plugged into the counter and the CD4 cells were counted (Ekaidem *et al.*, 2010)

3. RESULT

The result of the phytochemical analysis of aqueous leaf extract of *Ocimum basilicum* showed that the leaf extract contained alkaloids, saponins, flavonoids and carbohydrate in varying amounts while tannins were absent (table 1).

Table 1: Phytochemical constituents of aqueous leaf extracts of *Ocimum basilicum*

Constituents	Aqueous Solvent (mg/ml)
Alkaloids	++
Carbohydrate	+
Flavonoids	++
Saponins	++
Tannins	-

Key: ++ = present in high amount; + = present in moderate amount; - =absent

The mean values of the effect of aqueous extract of the leaves of *Ocimum basilicum* on haematological parameters is presented in Table 2. The results suggest that the administration of the leaf extract improved the haematological components in normal and immunocompromised rats. There was a significant doses dependent increase in WBC counts ($P<0.05$) in the normal and immunosuppressed rats when compared to the positive and negative control groups. Significant difference ($P<0.05$) was also observed in PCV count in the cyclophosphamide treatment groups when compared to the negative control. There was however no significant increase in HGB, RBC, Lymphocyte and granulocyte counts ($P>0.05$). Groups F (600mg/kg OBE), C (600mg/kg OBE+CTX) and E (400mg/kg OBE) treatment rats had WBC counts of $12.28\pm 0.73\%$, $13.65\pm 0.57\%$ and $10.90\pm 0.36\%$, lymphocyte counts of $63.55\pm 9.82\%$, $62.65\pm 8.45\%$ and $52.88\pm 1.65\%$ were recorded in Groups A (200mg/kg OBE+CTX), F(600mg/kg OBE) and D(200mg/kg OBE), while RBC counts of 6.53 ± 0.22 , 6.63 ± 0.22 and 6.48 ± 0.21 were recorded in Groups E, C and B respectively.

Table 2: Effect of *Ocimum basilicum* leaf extract on haematological parameters

Haematological Parameters	Group A (200mg/kg OBE+CTX)	Group B ((400mg/kg OBE+CTX))	Group C (600mg/kg OBE+CTX)	Group D (200mg/kg OBE)	Group E (400mg/kg OBE)	Group F (600mg/kg OBE)	Group G (Negative control)	Group H (Positive control)
WBC (%)	08.48 ± 0.28^b	09.75 ± 0.52^{bc}	13.65 ± 0.57^a	09.20 ± 0.08^b	10.90 ± 0.36^{cd}	12.28 ± 0.73^{de}	06.43 ± 0.05^b	04.14 ± 0.74^a
HGB (g/100ml)	13.90 ± 0.74^a	12.95 ± 1.03^a	13.00 ± 0.41^a	12.87 ± 0.74^a	12.90 ± 0.70^a	11.8 ± 1.18^a	13.43 ± 0.33^a	12.80 ± 1.11^a
PCV (%)	57.23 ± 3.92^b	58.25 ± 3.35^b	63.00 ± 0.49^b	57.37 ± 4.56^b	57.33 ± 4.48^b	56.64 ± 3.88^b	55.00 ± 3.17^{ab}	44.15 ± 4.78^a
Lymphocyte (%)	63.55 ± 9.82^a	57.62 ± 3.19^a	53.25 ± 0.68^a	52.88 ± 1.65^a	54.85 ± 0.13^a	62.65 ± 8.45^a	52.22 ± 1.52^a	46.45 ± 1.75^a
Granulocyte (%)	17.88 ± 4.84^a	20.38 ± 2.55^a	30.93 ± 4.70^a	26.17 ± 5.08^a	23.77 ± 3.39^a	22.02 ± 7.93^a	22.02 ± 2.33^a	20.70 ± 3.36^a
RBC(Million/ μ l)	6.36 ± 0.42^a	6.48 ± 0.21^a	6.63 ± 0.22^a	6.47 ± 0.21^a	6.53 ± 0.22^a	6.42 ± 0.21^a	5.51 ± 0.18^a	4.03 ± 0.14^a

OBE= *Ocimum basilicum* extract; CTX= cyclophosphamide

Values are mean \pm SEM

Mean values in the same row followed by a different letter differ significantly ($P<0.05$)

The result of aqueous leaf extract of *Ocimum basilicum* on CD4, humoral antibody response and phagocytic index (macrophage clearance) is given in Table 3. Administration of the leaf extract significantly ($P<0.05$) improved CD4 counts in the treatment groups when compared with the control groups. The extract produced CD4 counts of 10.00 ± 0.70 , 13.5 ± 0.95 and 09.50 ± 0.28 in Group F (600mg/kg OBE) Group C (600mg/kg OBE+CTX) and Group B (400mg/kg OBE+CTX) respectively. Administration of the leaf extract also significantly ($P<0.05$) improved the humoral antibody response of the rats in Group C (7.11 ± 0.30) and Group F (11.21 ± 0.23) when compared to the positive (3.95 ± 0.31) and negative (2.12 ± 0.22) controls. Although, result data showed that there were increases in the phagocytic index of the treatment groups, however there was no significant effect ($P> 0.05$) in the phagocytic activity across all studied doses when compared to the controls.

Table 3: Effect of aqueous leaf extract of *Ocimum basilicum* on CD4, humoral antibody response and phagocytic index

Parameters	Group A (200mg/kg OBE+CTX)	Group B (400mg/kg OBE+CTX)	Group C (600mg/kg OBE+CTX)	Group D (200mg/kg OBE)	Group E (400mg/kg OBE)	Group F (600mg/kg OBE)	Group G (Negative control)	Group H (positive control)
CD4(μ l)	07.25 \pm 0.25 ^{bc}	09.50 \pm 0.28 ^c	13.5 \pm 0.95 ^d	07.25 \pm 0.47 ^{bc}	8.50 \pm 0.86 ^c	10.00 \pm 0.70 ^c	6.50 \pm 0.28 ^b	3.00 \pm 0.57 ^a
Phagocytic index	0.052 \pm 0.001 ^a	0.059 \pm 0.001 ^a	0.059 \pm 0.001 ^a	0.065 \pm 0.005 ^a	0.066 \pm 0.006 ^a	0.073 \pm 0.004 ^a	0.037 \pm 0.002 ^a	0.169 \pm 0.14
Humoral antibody response	3.88 \pm 0.26 ^a	4.23 \pm 0.21 ^a	7.11 \pm 0.30 ^{ab}	4.00 \pm 0.29 ^a	4.98 \pm 0.27 ^a	11.21 \pm 0.23 ^b	3.95 \pm 0.31 ^a	2.12 \pm 0.22 ^a

OBE= *Ocimum basilicum* extract; CTX= cyclophosphamide

Values are mean \pm SEM

Mean values in the same row followed by a different letter differ significantly (P<0.05).

4. DISCUSSION

Medicinal plants and their products have been used for many centuries to treat different kinds of acute and chronic diseases. Immunomodulation is a procedure which can alter the immune system of an organism by interfering with its functions. Several studies have reported the immunomodulatory properties of plant phytochemical on immune cells and their products. Against this background *Ocimum basilicum* was investigated for its immunomodulatory properties. The dried powder was extracted with water and tested for its effect on haematological parameters, CD4 cells, humoral antibody response and phagocytic index (carbon clearance) in albino rats.

Hematological studies easily reveal anomalies in body metabolic processes, and the blood profile usually furnishes vital information on the response of the body to injury, deprivation and/or stress (Raza *et al.*, 2002). The result of the effect of *Ocimum basilicum* on haematological parameters showed that administration of the leaf extract significantly improved (P<0.05) the WBC component in the normal and immunosuppressed rats when compared to the positive and negative control groups. Significant difference (P<0.05) was also observed in PCV count in the cyclophosphamide treatment groups when compared to the negative control. The packed cell volume (PCV) is a measure of relative mass of blood. This effect could be due to the presence of high amount of alkaloids and flavonoids in the leaves of *Ocimum basilicum* as revealed by the results of phytochemical analysis. The increase might also be attributed to the homeostatic response by the endogenous defense system to the adverse effects of *O. basilicum*. There was however no significant increase in HGB, RBC, Lymphocyte and granulocyte counts (P>0.05). The absence of significant changes on these blood indices may suggest that the extract is safe in the rats with no deleterious effect on the haematological parameters. The result is similar to the findings of Ephraim *et al.* (2000), where significant higher values of WBC counts in the tested groups when compared with the control group was recorded. Also, Okon *et al.* (2011) reported that administration of aqueous leaf extract of *O. gratissimum* to albino rats did not have any significant effect on RBC, PCV and Hb.

The phagocytic activity of the reticuloendothelial system is generally measured by the rate of removal of carbon particles from the blood stream. The role of phagocytosis also involves the removal of microorganisms and foreign bodies, dead or injured cells. Increase in the carbon clearance index reflects the enhancement of the phagocytic function of mononuclear macrophage and nonspecific immunity. Phagocytosis by macrophages is important against parasites and its effectiveness is markedly enhanced by the opsonisation of parasites with antibodies and complementing C3b, leading to a more rapid clearance of parasites from the blood (Pallabi *et al.*, 1998). Although, result data from this study showed that there were increases in the phagocytic index of the treatment groups, however there was no significant difference (P> 0.05) in the phagocytic activity (index) across all studied doses when compared to the positive and negative controls. Contrary to this finding, Neelam and Nilofer (2010) reported in their study that aqueous and ethanolic leaf extract of *Ocimum basilicum* at the dose of 400 mg/kg showed significant (p<0.01) increase in phagocytic index when compared to control group.

The result of this study also revealed that administration of the leaf extract significantly (P<0.05) improved CD4 counts in the treatment groups when compared with the control groups. CD4 T cells play a central role in immune protection. They

do so through their capacity to help B cells make antibodies, to induce macrophages to develop enhanced microbicidal activity, to recruit neutrophils, eosinophils, and basophils to sites of infection and inflammation, and, through their production of cytokines and chemokines, to orchestrate the full panoply of immune responses. The humoral immunity involves interaction of B cells with the antigen and their subsequent proliferation and differentiation into antibody-secreting plasma cells. Antibody functions as the effector of the humoral response by binding to antigen and neutralizing it or facilitating its elimination by cross-linking to form clusters that are more readily ingested by phagocytic Cells (Benacerraf 1978). The effect of *Ocimum basilicum* leaf extract on humoral antibody response was also tested on sheep erythrocyte specific HA titre in albino rats. Cyclophosphamide showed significant inhibition in antibody titre response, while aqueous leaf extract of *Ocimum basilicum* counteract the suppression responses induced by cyclophosphamide. This indicates the enhanced responsiveness of macrophages, T and B lymphocyte subsets involved in antibody synthesis as there was significant differences ($P < 0.05$) in the HA titre of the treatment groups at the dose of 600mg/kg when compared to the control. Again, this effect may be attributed to the high amount of flavonoids present in the leaf extract. This is also similar to the findings of Neelam and Nilofer (2010) where aqueous and ethanolic leaf extract of *Ocimum basilicum* at the dose of 400 mg/kg showed significant ($p < 0.01$) effect of HA titre in both primary and secondary responses.

The indiscriminate use of antibiotics has fomented the emergence of bacterial resistance to commonly used drugs and, consequently, the need (and search) for new products that can replace those which are no longer effective.

5. CONCLUSION

This study has provided evidence that aqueous leaf extract of *Ocimum basilicum* when administered orally to albino rats exert considerable immunomodulatory activity, increasing white blood cell (WBC) counts, PCV, humoral antibody response (HA) and CD4 count. It is important to note that these effects were sufficient to increase both, the WBC count and the HA, to normal levels, in rats which were immunosuppressed by cyclophosphamide. This modulation of immunological parameters may offer promising therapeutic benefits exerted by the oral administration of the phytochemical compounds present in the leaves. Further investigations may be conducted to elucidate the mechanism by which *Ocimum basilicum* extract produced the observed changes on the hematopoietic system, and offer additional information on how B cells are activated, with the subsequent increase in antibody production.

REFERENCES

- [1] B. Benacerraf, "A hypothesis to relate the specificity of T lymphocytes and the activity of I region specific Ir genes in macrophages and B lymphocytes", *Journal of Immunology* Vol. 120, pp. 1809-1812, 1978.
- [2] S. Dash, L.K. Nath, S. Bhise, P. Kar, and S. Bhattacharya, "Stimulation of immune function activity by the alcoholic root extract of *Heracleum nepalense*", *Indian Journal of Pharmacology* Vol. 38, No. 5, pp. 336- 340, 2006.
- [3] C. Ekaidem, F. Galvano, L. Pierdomenico, E. Speroni, and M.C. Guerra, "Effects of rosmarinic acid against aflatoxin B1 and ochratoxin-A-induced cell damage in a human hepatoma cell line (Hep G2)", *Journal of Applied Toxicology*, Vol. 24 No. 4, pp. 289-296, 2004.
- [4] K.D. Ephraim, H.A. Salami, T.S. and Osewa, "The effect of aqueous leaf extract of *Ocimum gratissimum* on haematological and biochemical parameters in rabbits", *African Journal of Biomedical Research* Vol. 3, pp. 175-199, 2000.
- [5] V. Gayathri, V.V. Asha, and A. Subramoniam, 2005. "Preliminary studies on the immuno-modulatory and antioxidant properties of *Selaginella* species", *Indian Journal of Pharmacology* Vol. 7, pp. 381-385, 2000.
- [6] A.B. Gokhale, A.S. Damre, M.N. and Saraf, "Investigations into the immunomodulatory activity of *Argyreia Speciosa*. *Journal of Ethnopharmacology* 2003) Vol. 84, pp. 109-114, 2003.
- [7] A.A. Johrapurkar, M.M. Wanjari, S.P. Zambad, and S.N. Umathe, "Pyrogallol: A novel tool for screening immunomodulators", *Indian Journal of Pharmacology* Vol. 36, pp. 355-359, 2004.
- [8] M. Kivilompolo, and T. Hyötyläinen, "Comprehensive two-dimensional liquid chromatography in analysis of Lamiaceae herbs: characterisation and quantification of antioxidant phenolic acids" *Journal of Chromatography* Vol. 1145, No. 1, pp. 155-164, 2007.

International Journal of Novel Research in Life Sciences

 Vol. 5, Issue 3, pp: (19-25), Month: May - June 2018, Available at: www.noveltyjournals.com

- [9] C.K. Kokate, A.P. Purohit, and S.B. Gokhale, Pharmacognosy. Immunomodulators, Adaptogens and Rasayana, Analytical pharmacognosy, 17th edition. Pune: Archana enterprises; pp.586-588, 2001.
- [10] L.D. Neelam, and S.N. Nilofer, "Preliminary immunomodulatory activity of aqueous and ethanolic leaves extracts of *Ocimum basilicum* Linn in mice", International Journal of PharmTech Research Vol. 2, No. 2, pp. 1342-1349, 2010.
- [11] U.A. Okon, S.O. Ita, C.E. Ekpenyong, K.G. Davies, and O.I. Inyang, "Reduction of platelet and lymphocyte counts and elevation of neutrophil counts in rats treated with aqueous leaf extract of *Ocimum gratissimum*". African Journal of Biochemistry Research, Vol. 5, No. 9, pp. 303-306.
- [12] O.E. Ola-Davies, S.G. Olukole, O.A. and Amoo, Haematological and serum biochemical variables in rats treated with ethanol extract of the root of *Moringa oleifera*". African Journal of Biomedical Research, Vol. 17, pp. 31-35, 2014.
- [13] D.E. Pallabi, S.C. Dasgupta, and A. Gomes, "Immunopotentiating activity of Immu-21: A polyherbal product", Indian Journal of Pharmacology, Vol. 30, pp. 163-168.
- [14] M. Raza, O.A. Al-Shabanah, T.M. El-Hadiyah, and A.A. Al-Majed, "Effect of prolonged vigabatrin treatment on haematological and biochemical parameters in plasma, liver and kidney of Swiss albino mice", Scientia Pharmaceutica Vol. 70, pp. 135-145, 2002.
- [15] N. Roshan, and P. Savitri, "Review on chemical constituents and parts of plants as immunomodulators", Resource Journal of Pharmaceutical Biology and Chemistry Science" Vol. 4, pp. 76-89, 2013.
- [16] G.E. Trease, and M.C. Evans, *Textbook of Pharmacognosy*, 12th edition. Balliere, Tindall, London, pp. 343-383, 1983.
- [17] H. Wagner, "Search for plant derived natural products with immunostimulatory activity (recent advances)". Pure and Applied Chemistry Vol. 62, No. 7, pp. 1217-1222.