Aqueous extract of leaves of *Moringa oleifera* increased serum immunoglobulin G concentration in mice

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Abstract

Infectious diseases of public health importance such as tuberculosis, HIV/AIDS, as well as immunodeficiency disorders, and cancers have strong associations to immune status of individuals. Therefore, drugs that modulate immune response can be beneficial in the prevention and management of these health disorders. This study was aimed at determining the effect of aqueous extract of leaves of *Moringa oleifera* on plasma IgG and total WBC count in mice. Twenty mice were divided into 5 groups of 4 animals per group. The groups (1, 2, 3, 4, 5) were given immunostimulatory drugs and extract orally as follows: 1 (extract 10 mg/kg); 2 (extract 20 mg/kg), 3 (leaf extract 40 mg/kg), 4 (levimasole 1 mg/kg); 5 (normal feeds only) all for 21 days. Thereafter, sera from the mice were analysed for serum IgG and WBC count. Data was analysed for statistical significance using analysis of variance (ANOVA) plus Bonferroni's Multiple comparison. Mean values (± SD) of total WBC count (x 10^9/L) were 9.65 ±3.35, 9.85 ±4.13, 9.00 ±1.31, 7.03 ±1.32 and 8.18 ±2.44 for groups 1, 2, 3, 4, 5 respectively while mean values (±SD) of serum IgG (µg/dl) were 1333.35±2205, 1072.00 ± 86.43, 1168.90 ±77.83, 1420.23 ± 135.49 and 1665.88 ± 167.72 for groups 1, 2, 3, 4 and 5 respectively. P values for total WBC and serum IgG were 0.640 and 0.000 respectively (level of significance = 0.05). Oral administration of extract of leaves of *Moringa oleifera* significantly increased the serum concentration of IgG but did not increase the total WBC count in mice.

Keywords: Extract; IgG; Immune response; Levamisole; Mice; *Moringa oleifera*; WBC

1. Introduction

Microorganisms abound in our environment. These eventually gain entrance to our bodies through the skin and natural orifices on our body such as the mouth, the nostrils, the eyes, and the external auditory meatus.

The body responds to this invasion by microbes and foreign substances by mounting immune responses. The cellular component of this response involves the proliferation of immune competent cells that take part in immune response while the humoral component involves the production of antibodies in the form of immunoglobulins (IgG, IgA, IgM, IgE, IgE).

The IgG class is the most abundant. It has a plasma concentration of 10-15 ng/ml and constitutes 75% of human plasma immunoglobulins. It is mainly involved in humoral immune responses through agglutination, opsonisation, complement activation, and antibody dependent cell mediated cytotoxicity (ADCC). In addition to being the only transplacental antibody, it is also mainly involved in secondary immune response because it is produced after class switching generated by primary immune response. Studies have shown that all components of the immune system are affected
changes [1, 2]. In particular, the serum concentration of IgG increases with age, probably as a result of increasing infections and consequent primary and secondary immune responses [3].

The white blood cells (WBC) are referred to as soldiers of the body as they are involved in inflammatory and immune responses. They are the cells most involved in immunity and immune response. In particular, the lymphocytes, through the B- and T-cells, mediate humoral and cell-mediated immune responses. The monocyte/macrophages are responsible for maintaining natural immunity. The basophils and mast cells (tissue-fixed) are involved in type 1 hypersensitivity reactions. Therefore, the role of the white blood cells in immunity and allergy cannot be overemphasized.

Certain disease conditions or physiological states can result in defective immunity. For example, alcoholism, diabetes mellitus, obesity, malnutrition, chronic stress can result in defective immune response [4, 5]. Also, the immune response is diminished in both extremes of age due to prematurity and decline in functions of the body organs respectively [6, 7]. Infectious diseases of public health importance such as tuberculosis, HIV/AIDS, malaria, childhood killer diseases are affected by the immune status of the individual. Also, non-infectious diseases such as immunodeficiency disorders, graft versus tissue reactions, and even cancers have strong associations to immune status of individuals. Therefore, drugs or supplements that boost immune response can be beneficial in the prevention and management of these health disorders.

1.1. Justification for the study

Drug treatment of immunity-related disorders requires the use of immunomodulatory drugs (immunostimulants or immunosuppressants depending on the nature of the disorder). These immunomodulators have their drawbacks; some are toxic, others are costly and are therefore beyond the reach of the average citizen. Therefore, there is the need to find cheaper and safer alternatives.

*Moringa oleifera* is a medicinal plant whose leaves are also used as vegetables. Previous studies have demonstrated its anti-inflammatory and hepatoprotective properties [8, 9]. Gupta et al. studied the immunomodulatory effects of *Moringa oleifera* leaves on normal and immunosuppressed mice models and found that pre-treatment with this extract inhibited cyclophosphamide-induced bone marrow suppression in mice [10].

Another study revealed that aqueous leaf extract of *Moringa oleifera* produced dose-dependent increases in total white blood cells count in Wistar albino rats and mean haemagglutination antibody titre to sheep red blood cells. [11]. Furthermore, Sreelatha and Padma demonstrated significant antioxidant activity of this extract against free radicals [12].

The study was therefore to determine the effect of the aqueous extract of the leaves of this plant on immune response in mice. The study was based on immunoglobulins because they are important mediators of humoral immune response. In particular, measurement of plasma IgG was chosen because it constitutes about 75% of circulating immunoglobulins.

1.2. Objectives of the study

The general objective of the study was to find out if the aqueous leaf extract of *Moringa oleifera* can boost immune response in mice. Specific objectives included:

- To determine the effect of escalating doses of aqueous extract of leaves of *Moringa oleifera* on serum IgG and WBC counts in mice.
- To compare the effect of the extract and that of levamisole (immunostimulant) on serum IgG in mice.
- To compare the effect of the extract and that of levamisole on WBC count in mice.

1.3. Hypotheses

The null hypothesis stated that aqueous leaf extract of *Moringa oleifera* does not increase serum IgG and total WBC counts in albino mice.

The alternative hypothesis stated that aqueous leaf extract of *Moringa oleifera* increases serum IgG and total WBC counts in albino mice.

The null hypothesis was tested at a significance level (P-value) of 0.05. It would be rejected if the p value is < 0.05 and accepted if p value > 0.05.
2. Materials and methods

2.1. Study area
This study was conducted at the animal house, Department of Human Physiology, Faculty of Basic Medical Sciences, College of Medicine, Chukwuemeka Odumegwu Ojukwu University, Uli campus. The study was conducted in accordance with guidelines for the care and use of laboratory animals [13].

2.2. Sample size determination
A sample size of 20 was chosen using the Resource Equation Method [14]. There was no correction for attrition.

2.3. Animal source
Twenty (20) male mice, 6-8 weeks old, average weight 35 grammes, 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 6 mice was housed in a metal cage measuring 60cm X 45cm X 30cm and was allowed free access to animal feeds (Top Feeds, Nigeria) and clean drinking water. Left over feeds and water were discarded and the cages was properly cleansed with anti-septic solution of chlorohexidine every 12 hrs. Artificial light was provided by fluorescent lamp (Phillips Holland, 18 watts) and light-dark cycle of 12-12 hrs was maintained. The animals were acclimatized for two weeks before the commencement of study.

2.4. Preparation of plant extracts
Three (3) kilograms of fresh leaves of *Moringa oleifera* was collected, washed and 315 grammes of the dried leaves was ground into fine powder and 32 grammes of the powder was extracted with 300ml of distilled water using the Soxhlet method.

The percentage yield of the extract was calculated using the following formula:

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

2.5. Experimental procedure
The mice were randomly divided into 5 groups of 4 each. Thereafter, we treated the animals with the following drugs by oral administration for 21 days [15] as follows:

- Group 1 (Positive control, PC): Each mouse was given Levamisole 0.5 mg/kg. This was used as the positive control.
- Group 2 (Negative control NC): Each mouse was given normal feed (no drugs, no extract). This was used as the negative control.
- Group 3 (Low dose, LC): Each mouse was given 10 mg/kg aqueous leaf extract of *Moringa oleifera*.
- Group 4 (Medium dose, MD): Each mouse was given 20 mg/kg aqueous leaf extract of *Moringa oleifera*.
- Group 5 (High dose, HD): Each mouse was given 40 mg/kg aqueous leaf extract of *Moringa oleifera*.

A treatment chart was maintained. After 21 days, blood samples were collected from each mouse for the determination of serum IgG and total WBC counts.

2.6. Calculation of doses
Doses of extract for the different groups of mice were calculated as follows:

Group 3 (Low dose, LC): the dose of extract was 10 mg/kg; average weight of mice was 35 grammes.

From the dose 10 mg/kg, 1000 gm b.w. receives 10 mg extract

\[
35 \text{ gm b.w. will receive } (35/1000) \times (10/1) = 0.35 \text{ gm (350 mg)}
\]

100 gm b.w. animal will receive drug (or extract) in 1 ml solvent [16].
So, 35 gm b.w. mouse will receive extract dissolved in \((35/100)\) ml = 0.35 ml distilled water.

So each mouse in group 3 received 0.35 gm extract dissolved in 0.35 ml distilled water.

The same calculation was done for groups 4 and 5 and the corresponding weights of extract and volumes of distilled water administered to the mice.

2.7. Collection of blood samples
Each mouse was anesthetized using intramuscular ketamine and diazepam at 50 mg/kg and 5 mg/kg, respectively [17].

Subsequently, blood samples were collected as described by Hoff [18]. Part of the withdrawn blood samples were transferred gently into plain specimen bottles and allowed to coagulate. The separated sera were collected for the determination of serum IgG. The rest were transferred into plain bottles containing anticoagulant ethylenediaminetetraacetic acid (EDTA) for total WBC counts.

We did ELISA-based assays for serum IgG according to the manufacturer's instructions (Life Diagnostics, West Chester, USA). Subsequently, the optical densities obtained were converted into mg/ml using digital software, Gen 5 (Diagnostic Automation, USA), a component of the microplate reader (Model No DAR 8000. Diagnostic Automation Inc., USA). Total WBC count was done using Haematology Autoanalyser (Erma Inc, PCE - 210).

2.8. Statistical analysis
The mean values (±SEM) for the generated data were calculated. Test of statistical significance for data from test and control groups of mice was done for total WBC and serum IgG using analysis of variance (ANOVA) followed by Bonferroni multiple comparisons. All statistical tests were done using SPSS version 21 and the results were taken as statistically significant at \(P < 0.05\).

3. Results
None of the mice died during the course of the study.

3.1. Yield of the extract
Three hundred and fifteen (315) grammes of the dried leaves yielded 32 grammes of the extract giving a percentage yield of 10.2

3.2. Total WBC count and serum IgG values
The mean values for total WBC and serum IgG are shown in Table 1 and Figures 1 and 2. The mean serum IgG values for the groups fell within the normal values of 100-1500 µg/dl (1-1.5 mg/dl) except that of group 4 (high dose) which showed a higher value (Table 1). The mean serum IgG values of the groups that received the extract (groups 3, 4, 5) were higher than those of the negative control group. Using analysis of variance (ANOVA) this increase was statistically significant as shown by the \(p\)-value of 0.00 (Table 2). Bonferroni multiple comparison of the groups’ serum IgG values revealed that the statistical significance lay between the values of high dose (HD) group versus negative control (NC) group and those of high dose (HD) group versus low dose (LD) group with \(p\)-values of 0.000 and 0.002 respectively.

The extract at the dose of 20 mg/kg (medium dose) produced reduction of the total WBC count compared to other doses and the controls but this reduction was not statistically significant (Tables 1, 2; Figure 1).
Table 1. Mean (± SEM) total WBC (x 10⁹ /L) and serum IgG (µg/dl) after 21 days of oral administration of different doses of aqueous extracts of leaves *Moringa oleifera* and 0.5 mg/kg levamisole to test and control groups of mice respectively.

| Groups           | WBC (x 10⁹/L)   | IgG (µg/dl)      |
|------------------|-----------------|------------------|
| Positive control | 9.65±3.35       | 1333.35±220.51   |
| Negative control | 9.58±4.13       | 1072.00±86.83    |
| Low dose         | 9.00±1.31       | 1168.90±77.83    |
| Medium dose      | 7.03±1.32       | 1420.23±135.49   |
| High dose        | 8.18±2.44       | 1665.85±167.72   |
| F-test           | 0.643           | 9.824            |
| P-value          | 0.640           | 0.000            |

Table 2. Analysis of variance (ANOVA) for total WBC (x 10⁹ /L) and serum IgG (µg/dl) obtained after 21 days of oral administration of different doses of aqueous extracts of leaves of *Moringa oleifera* and 0.5 mg/kg levamisole to test and control groups of mice respectively.

| ANOVA          | Sum of squares | Df | Mean squares | F-test | P-value |
|----------------|----------------|----|--------------|--------|---------|
| WBC            | Between groups | 19.353 | 4 | 4.838 | 0.643 | 0.640 |
|                | Within groups  | 112.893 | 15 | 7.526 |       |        |
|                | Total          | 132.246 | 19 |      |       |        |
| IgG            | Between groups | 85383.297 | 4 | 213459.574 | 9.824 | 0.000 |
|                | Within groups  | 325917.785 | 15 | 21727.852 |       |        |
|                | Total          | 1179756.082 | 19 |      |       |        |

Figure 1. Bar chart showing total WBC (x 10⁹ /L) of mice treated with increasing doses of aqueous leaf extract of *Moringa oleifera* compared with those of mice treated with 0.5 mg/kg levamisole (positive control) and no drugs (negative control). Low dose (10 mg/kg extract), Medium dose (20 mg/kg extract), High dose (40 mg/kg extract).
Table 3 Multiple comparisons (Bonferroni) of serum IgG values (µg/dl) obtained after 21 days of oral administration of different doses of aqueous leaf extract of *Moringa oleifera* and levamisole to test and control groups of mice respectively.

| Dependent Variable | Group | (I) Group | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval |
|--------------------|-------|-----------|-----------------------|------------|------|-------------------------|
|                    |       |           |                       |            |      | Lower bound              | Upper bound |
| IgG                |       |           |                       |            |      |                         |             |
|                    | Group 1 |          |                       |            |      |                         |             |
|                    | Group 2 |          | 261.3500              | 104.23016  | 0.241| -81.1543                | 603.8543    |
|                    | Group 3 |          | 164.4500              | 104.23016  | 1.000| -178.0543               | 506.9543    |
|                    | Group 4 |          | -86.87500             | 104.23016  | 1.000| -429.3793               | 255.6293    |
|                    | Group 5 |          | -332.52500            | 104.23016  | 0.061| -675.0293               | 9.9793      |
|                    | Group 2 |          | -261.3500             | 104.23016  | 0.241| -603.8543               | 81.1543     |
|                    | Group 3 |          | -96.90000             | 104.23016  | 1.000| -439.4043               | 245.6043    |
|                    | Group 4 |          | -348.22500            | 104.23016  | *0.045| -690.7293              | -5.7202     |
|                    | Group 5 |          | -593.87500            | 104.23016  | *0.000| -936.3793              | -251.3707   |
|                    | Group 3 |          | 164.45000             | 104.23016  | 1.000| -506.9543               | 178.0543    |
|                    | Group 4 |          | 96.90000              | 104.23016  | 1.000| -245.6043               | 439.4043    |
|                    | Group 5 |          | 251.32500             | 104.23016  | 0.292| -593.8293               | 91.1793     |
|                    | Group 4 |          | 496.97500             | 104.23016  | *0.000| -839.4793              | 154.4707    |
|                    | Group 5 |          | 86.87500              | 104.23016  | 1.000| -255.6293               | 429.3793    |
|                    | Group 2 |          | 348.22500             | 104.23016  | *0.045| 5.7207                  | 690.7293    |
|                    | Group 3 |          | 251.32500             | 104.23016  | 0.292| -91.1793                | 593.8293    |
|                    | Group 4 |          | -245.65000            | 104.23016  | 0.324| -588.1543              | 96.8543     |
|                    | Group 5 |          | 332.52500             | 104.23016  | 0.061| -9.9793                 | 675.0293    |
|                    | Group 2 |          | 593.87500             | 104.23016  | 0.000| 251.3707                | 936.3793    |
|                    | Group 3 |          | 496.97500             | 104.23016  | 0.002| 154.4707                | 839.4793    |
|                    | Group 4 |          | 245.65000             | 104.23016  | 0.324| -96.8543                | 588.1543    |

*: The mean difference is significant at the 0.05 level (p-value ≤ 0.05)

Figure 2 Bar chart showing mean serum IgG (µg/dl) of mice treated with increasing doses of aqueous leaf extract of *Moringa oleifera* compared with those of mice treated with 0.5 mg/kg levamisole (positive control) and no drugs (negative control). Low dose (10 mg/kg extract), Medium dose (20 mg/kg extract), High dose (40 mg/kg extract).
4. Discussion

Oral administration of the extract to mice produced statistically significant increase in serum concentration of IgG. Bonferroni multiple comparison of the groups' serum IgG values revealed that the statistical significance lay between the values of high dose (HD) group versus negative control (NC) group and those of high dose (HD) group versus low dose (LD) group with p-values of 0.000 and 0.002 respectively. This shows that the effect of the extract on the serum concentration of IgG is dose dependent. The effect of this extract on serum immunoglobulin G is similar to that of some previous studies. For instance, Obi et al. found that oral administration of the leaf extract stimulated the production of IgA, IgM and IgG [19].

Other researches also support the immunomodulatory properties of this extract. The extract has been found to increase total WBC count, neutrophil count, and macrophage phagocytosis [20]. The extract equally stimulates the growth and development of the primary lymphoid organs (thymus and spleen) which play important roles in innate and acquired immunity [21]. It also increases antioxidant enzymes superoxide dismutase (SOD) and glutathione peroxidise which has complementary roles in immune mediation [22].

In this study, this extract increased serum IgG concentration even without prior drug induced immunosuppression. This means that the extract will increase baseline immunity in persons without immunosuppression and boost immune status in persons with compromised immunity.

In this study the extract at the dose of 20 mg/kg (medium dose) produced reduction of the total WBC count compared to other doses and the controls but this reduction was not statistically significant. This contrasts with findings in other studies where oral administration of the extract increased the total WBC count in small laboratory animals [19, 23]. The differences in WBC findings could be because in such studies the administrations of the extract were preceded by cyclophosphamide induced immunosuppression [23]. This drug-induced immunosuppression could lead to opportunistic infections which could increase total WBC count not related to the effect of the extract. Also the drug induced immunosuppression could lead to reactionary leucocytosis unrelated to opportunistic infections [24]. Besides, leucocytosis may also result from physical or emotional stress which can occur if the laboratory animals are not handled in line with standard laboratory procedures.

Previous studies have equally demonstrated that oral administration of this extract modulates both humoral and cellular immunity in the recipient laboratory animals. Such studies attributed the immunomodulatory properties of this extract to the presence of micronutrients such as copper, selenium, zinc, vitamin C, vitamin B6, manganese, etc which important in the proliferation and maturation of B- and T-lymphocytes [25, 26]. In particular, the T-lymphocytes produce both immunostimulatory (IL-1, IL-2, IL-4) and immunosuppressive (IL-10, IFN-γ) cytokines which are important modulators of the immune system.

5. Conclusion

At the doses and concentrations used in this study, aqueous extracts of leaves of Moringa oleifera produced statistically significant dose dependent increase of plasma IgG concentration in mice. In fact, oral administration of high dose of the extract produced higher serum concentration of IgG than orally administered levamisole (0.5 mg/kg), a standard immunostimulant. However, the effect of the extract on total WBC count was not statistically significant. Since the null hypothesis was tested at a significance level (p-value) of 0.05 and the significance test produced a p-value < 0.05 for serum IgG and > 0.05 for total WBC, the null hypothesis was rejected for serum IgG and accepted for total WBC count. This implied that this extract, when orally administered, increases serum IgG but does not increase total WBC count in mice. Since the leaves of this plant is edible and the extract has been established to be to be non-toxic to humans in previous studies, further studies on this extract should involve clinical trials on humans with natural or drug-induced immunodeficiency disorders.

Compliance with ethical standards

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Disclosure of conflict of interest
No conflict of interest to be declared.

Statement of ethical approval
The ethical approval for this study was obtained from the Ethical Committee, College of Medicine, Chukwuemeka Odumegwu Ojukwu University, Awka Campus and the study was conducted in accordance with guidelines for the care and use of laboratory animals [13]

References
[1] Globerson A, Effros RB. Ageing of lymphocytes and lymphocytes in the aged. Immunology Today. 2000; 21(10):515–521.
[2] Pomorska-Mól M, Markowska-Daniel I. Age-dependent changes in the relative and absolute size of lymphocyte subsets in the blood of pigs from birth to slaughter. Bulletin of Veterinary Institute in Pulawy, 2011; 55:305–310.
[3] Pomorska-Mól M, Krzysiak MK, Larska M, Włodarek J. The First Report of Immunoglobulin G, M, and A Concentrations in Serum of European Bison and their Changes with Age. Journal of Immunology Research, 2020.
[4] Krueger JM, Majde JA. Humoral links between sleep and the immune system: research issues. Annals of the New York Academy of Sciences. 2003; 992(1):9-20.
[5] Bryant PA, Trinder J, Curtis N. Sick and tired: does sleep have a vital role in the immune system? Nature Reviews Immunology. 2004; 4(6), 457-467.
[6] Chandra RK. Nutrition and the immune system: an introduction. The American Journal of Clinical Nutrition. 1997; 66(2):460S-463S.
[7] Aw D, Silva AB, Palmer DB. Immunosenescence: emerging challenges for an ageing population. Immunology. 2007; 120(4):435-446.
[8] Nikkon F, Saud ZA, Rahman MH, Haque ME. In vitro antimicrobial activity of the compound isolated from chloroform extract of Moringa oleifera Lam. Pak. J. Biol. Sci, 2003; 6: 1888-1890.
[9] Stohs SJ, Hartman MJ. Review of the Safety and Efficacy of Moringa oleifera. Phytother. Res. 2015; 29(6):796-804.
[10] Gupta R, Mathur M, Bajaj VK, Katariya P, Yadav S, Kamal R, Gupta R. Evaluation of antidiabetic and antioxidant activity of Moringa Oleifera in experimental diabetes. J Diabetes. 2012; 4(2):164-71.
[11] Nfambi J, Bbosa GS, Sembajwe LF, Gakunga J, Kasoo JN. Immunomodulatory activity of methanolic leaf extract of Moringa oleifera in Wistar albino rats. J Basic ClinPhysiolPharmacol. 2015; 26(6):603-611.
[12] Sreelatha S, Padma PR. Antioxidant activity and total phenolic content of Moringa oleifera leaves in two stages of maturity. Plant Foods Human Nutr. 2009; 64(4):303-11.
[13] Institute for Laboratory Animal Research, ILAR. Guide for the Care and Use of Laboratory animals. Washington DC, USA: National Academies Press. 1996.
[14] Ughachukwu PO. Fundamental Principles of Research in Basic Medical Sciences. Onitsha: Nigeria. Living Seed Publishers Limited. 2019.
[15] Ughachukwu PO, Nwozor CM, Nwafia IN. Effect of aqueous extract of Ocimum gratissimum on anti-retroviral drug induced hepatotoxicity in rats. European Journal of Medicinal Plants. 2015; 13(1): 1-11.
[16] Organization for Economic Cooperation and Development, OECD. OECD Guideline for testing of chemicals. OECD 420. 2001.
[17] Garguilo S, Greco A, Gramazini M, Esposito S, Affuso A, Brunette A, Vesce G. Mice anesthesia, analgesia, and care, Part1: anesthetic considerations in preclinical research. ILAR Journal. 2012; 53:E55-69.
[18] Hoff J. Methods of blood collection in the mouse. Lab Animal. 2000; 29: 47-53.
[19] Obi A, Egwurugwu JN, Ojefa SO, Ohamaeme MC, Ekweogu C, Ogunnaya FU. Immunomodulatory effects of hydromethanolic extract of Moringa oleifera leaf on male Wistar rats. Niger J Exp Clin Biosci. 2018; 6:26-32.
[20] El-Gawad EA, El-Asely AM, Soror EI, Abbass A. Effect of dietary Moringa oleifera on the immune response and control of Aeromonashydrophila infection in Nile tilapia (Oreochromis nilotica). Aquaculture International, 2020; 28: 389-402.

[21] Gupta A, Gautam MK, Singh RK, Kumar MV, Rao CV, Goe RK, Anupurba S. Immunomodulatory effect of Moringa oleifera Lam extract on cyclophosphamide-induced toxicity in mice. Indian J Exp Biol. 2010; 48:1157-1160.

[22] Campa-Cordova AI, Hernandez-Saavedra NY, Ascencio F. Superoxide dismutase as modulator of immune function in American white shrimp. Comparative Biochemistry and Physiology part C. 2002; 133:557-565.

[23] Nfambi J, Bhosa GS, Sembajwe LF, Gakunga J, Kasolo JN. Immunomodulatory activity of methanolic leaf extract of Moringa oleifera in Wistar rats. Journal of Basic and Clinical Physiology and Pharmacology. 2015; 26(6), 603-611.

[24] Drugs.com. High white cell count. 2018.

[25] Janeway CA, Travers P, Walport M, Shlomchik MJ. The immune system in health and disease: Immunobiology. New York: Garland Publishing. 2001.

[26] Rodrigo JM, Iwata M, Ulrich VA. Vitamin effects on the immune system: vitamins A and D take centre stage. Nat Rev Immunol. 2008; 8: 685-698.