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Effect of Moringa Oleifera Leaf Extract on Immunoglobulins of Albino Wistar Rats

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Abstract: ***The immune system is a host defense mechanism comprising many biological structures and processes within an organism that protects against disease. This study aims to determine the effect of aqueous extract of Moringa oleifera leaf on Immunoglobulin in Wistar rats. Twenty five (25) male rats were randomly divided into five (5) groups (A-E) of five (5) rats in each group. Group A served as control and were fed with conventional feed and distilled water only, while groups B, C, D and E served as test groups and received 20, 40, 60 and 80mg/kg body weight (bw) of the extract respectively. The administration was for 14 days. Thereafter groups B-E were inoculated with the test organisms and monitored for two weeks. At the end of administration, blood samples were collected by cardiac puncture and IgA, IgG and IgM levels were estimated by the immunoturbudrimetric method. Result obtained showed a significant (p<0.05) reduction in the serum concentration of immunoglobulin G (IgG) and a significant (p<0.05) increase in the serum levels of immunoglobulins A and M (IgA and IgM) at a dose of 40mg/kgbw of the extract. There was significant (p<0.05) increase in immunoglobulin A at 60mg/kgbw when compared with the control. There were no significant changes in the immunoglobulins at the other concentrations. The present study demonstrates possible beneficial therapeutic effect on the amelioration of immunological diseases such as systemic lupus erythematosus (SLE), rheumatoid arthritis, psoriasis and infections caused by Staphylococcus and Streptococcus organism respectively in human clinical trials, especially at low concentration for immunoglobulins A and M. This finding supports the anecdotal use of leaf extracts of Moringa oleifera as an immune boosting agent***

Keywords: *Moringa oleifera*, Immunoglobulin, Wistar Rats, immunomodulatory, test organisms

1. Introduction

The immunesystem is known to be the body's defense mechanism. It possesses irrefutable properties for protecting the body against various infections. These infections can be caused by various pathogens – bacteria, virus, toxins and parasites. The fundamental property of a healthy immune system is to identify the pathogens from the organism's own healthy tissues and destroy them. The immune system can also affect cell mutations and carcinogenic growths of the body (Muchenje, 2011).The adaptive immune response is designed to combat specific pathogens; typically, specialized white blood cells called lymphocytes attack the infection or intrusion. The body produces three types of lymphocytes, known as B-cells, T-cells and natural killer or NK cells. T-cells and B-cells are tasked with identifying and responding to specific threats to the body. This process is called antigen representation and allows the cells to produce specific responses to pathogens that the body has previously encountered. NK cells use a slightly different process and can defend against some pathogens to which the individual may not previously have exposed (Eme, 2013).Healthy immune systems are necessary in order to fend off disease and protect the body against toxins and pathogens. The immune system also reacts to mutations and cancerous growths within the body, typically attacking these cells with cytotoxic granules that contain powerful cell-killing enzymes. In all these cases, the value of the immune system depends on its ability to distinguish between the organism's own cellular structures and those of external pathogens. One way in which this is achieved is through immunological memory. Vaccinations are effective due to this ability of the immune system to remember and maintain active defenses against previously encountered bacteria and viruses, essentially destroying these pathogens before they can gain a foothold in the body (Fagnoni *et al.*, 2008).Immune modulation is the manipulation of immune response to suppress unwanted responses resulting from autoimmunity, allergy, and transplant rejection, and to stimulate protective responses against pathogens that largely elude the immune system. An immune modulator is any substance that affects directly or indirectly the immune response to external agents or therapeutics and prevents or reduces the development of degenerative diseases (Fagnoni *et al.*, 2008). They have broad effects on the entire immunity system, but affect primarily cell mediated immunity, while the humoral immunity may be affected indirectly (Goldsby *et al*., 2000). Immune modulators achieve their effects by boosting specific areas of the immune system, most especially, the innate immunity and the activities of T lymphocytes. They are known to have amplifier and suppressor activities, depending on the immune status of the user. Traditional medicine practitioners claim that some herbal preparations detoxify toxins in the body, cleanse the body of such toxins, and ultimately modulate the immune system (Oyewo *et al.*, 2012). Example of such plant is *Moringa oleifera*.

Several valuable reviews of the ethnobotanical uses of *M. oleifera* are available. *Moringa* has been found to be a good source of polyphenols and antioxidants (Hsu *et al.*, 2006). Phytochemicals such as vanillin, omega fatty acids, carotenoids, ascorbates, tocopherols, beta-sitosterol, moringine, kaempferol, and quercetin have been reported in its flowers, roots, fruits, and seeds. The leaves, in particular, have been found to contain phenolics and flavonoids; these compounds have various biological activities, including antioxidant, anticarcinogenic, immunomodulatory, antidiabetic, antiatherogenic, and hepatoprotective functions and the regulation of thyroid status. Moreover, leaves contain trace elements that are essential to human health. For instance, magnesium, iron, selenium, and zinc play an important role in metabolism, and interest in these elements is increasing together with reports relating trace element status and oxidative diseases (Anjorin*et al.*, 2010).

The consumption of the leaf of *Moringa oleifera* has been alleged to balance or boost the energetic, soothing ability, prevent ulcer, inflammation, pain, skin problems, detoxify

the blood and gastrointestinal tract, promote wound healing and promote immune functions. In Nigeria, leaf preparations of *Moringa oleifera* is widely used in folklore for the treatment of immune system related disorders (Anjorin*et al.*, 2010). In this study, the *invivo* survey of the immunological properties of *Moringa* leaves on Wistar rats infected with *Staphylococcus* and *Streptococcus* pathogens will be studied.

Staphylococci are Gram-positive bacteria, with diameters of 0.5 – 1.5µm and characterized by individual cocci, which divide in more than one plane to form grape-like clusters. To date, there are 32 species and eight sub-species in the genus Staphylococcus, many of which preferentially colonize the human body, however *Staphylococcus aureus* and *Staphylococcus epidermidis* are the two most characterized and studied strains (Wertheim *etal*., 2004).

The *Staphylococci* are non-motile, non-spore forming facultative anaerobes that grow by aerobic respiration or by fermentation. Most species have a relative complex nutritional requirement, however; in general they require an organic source of nitrogen, supplied by 5 to 12 essential amino acids, e.g. arginine, valine, and B vitamins, including thiamine and nicotinamide. Members of this genus are catalase-positive and oxidase-negative, distinguishing them from the genus *Streptococci*, which are catalase-negative, and have a different cell wall composition to *Staphylococci* (Winn-Washington *et al.*, 2006).

2. Materials and Methods

Collection of Plant

Fresh and healthy *Moringa* leaves were collected from the botanical garden of the Department of Botany, Ambrose Alli University, Ekpoma and were identified by the herbarium at the same Department.

Preparation of Leave Extracts

The leaves were thoroughly rinsed in tap water to remove any residual dirt, dried in an air-oven at 40°C for 14 days and then milled into fine powder. The powered leaf (100g) was subsequently extracted with 1000ml deionized water using Soxhlet apparatus. The resulting crude aqueous extract was filtered by passage through a Whatmann No. 3 filter paper followed by concentration in vacuole at 40°C using a rotary evaporator.

Source of Bacteria Isolates

Test bacteria used for this study were clinical isolates of species of *Staphylococcus* and *Streptococcus* from urine and stool samples of patients from Irrua Specialist Teaching Hospital (ISTH), Irrua, Edo State. The identity of the isolates were confirmed using the methods of colonial characters, Gram stain and biochemical tests.

Preparation/Inoculation of the Test Organisms

The different organisms isolated; *Staphylococcus* and *Streptococcus* species were sub-cultured into peptone water for 8 hours before inoculation. The animals were then inoculated with the organism orally through water.

Collection and Treatment of Test Populations

Twenty five (25) Adult Albino Wistar Rats weighing between 160g to 200g procured from the Animal Farm, College of Medical Sciences, Ambrose Alli University, Ekpoma, Edo State were used as population for this study. After procurement, the rats were transferred to the Histology Laboratory and were randomly divided into five groups comprising of five rats per group and designated groups A to E. The rats were allowed to acclimatization for two weeks and subsequently treated as follows: Group A, (which served as control) received grower's mash and water only while groups B, C, D and E received in addition 20, 40, 60 and 80mg/kg body weight of the extract respectively. The doses of the extract chosen were based on the result of acute toxicity (LD_{50}) study conducted by National Institute of Health, (2005), which was obtained as 1g/kg for aqueous leaf extract of *Moringa oleifera*. The extract was administered orally once daily between 8am and 9am using a cannula attached to a 2ml syringe for 14 days. Thereafter, groups B-E were inoculated with the test organisms and monitored for two weeks.

Detection of Immunoglobulins in serum of Experimental Animals

At the end of the experimental procedures, the rats were then fasted for 12 hours and body weights and temperature determined before they were sacrificed using 25% urethane (ethyl carbamate) at the dose of 0.6ml/100g body weight. Blood samples were obtained through cardiac puncture for analyses of immunoglobulin A, G and M using the immunoturbidimetric method and spectrophotometer.

The immunoglobulin reagent and the Chemwell auto-analyzer were pre-warmed to 37°C. Ten microlitre (10µl) of reagent was used for IgM, while 7µl was used for IgG and IgA respectively. This was added to 1ml of sample. The computer interphase attached to the Chemwell auto-analyzer was programmed to suite the analytical requirement and the process for analysis of IgA, IgG and IgM were undertaken according to predefined program. The results obtained were recorded in g/L.

3. Results

The results obtained from assessment of weight, temperature and observations of the wistar rats are as shown in Tables 1-2.

In Table 1 is shown the result of temperature, weight and observation of the Wistar rats. The result showed that there was no significant difference in the temperature and weight of the control (group A) before and after the experiment. On the other hand, there was an increase in temperature and decrease in weight of Wister Rats in group B. Groups C, D and E showed slight variability in the temperature and weight before and after the experiment but the differences were not significant. Finally, Groups A, C, D and E showed no physical symptoms after the experiment but group B showed withdrawal behaviour, hair loss and sore on the legs.

Table 2 showed the serum levels of Immunoglobulins following the administration of graded doses of *Moringa oleifera*leaf extract in Wistar rats. The results showed there

was a significant ($p<0.05$) increase in the serum level of immunoglobulin A (IgA) following the administration of 40mg/kg and 60mg/kg body weight of the extract when compared with the control. A non–significant reduction was recorded for the dose of 80mg/kg when compared with the control. However, there was no particular change in serum level of immunoglobulin A (IgA) when compared with the control.

A significant ($p<0.05$) reduction was recorded in the serum level of immunoglobulin G (IgG) for the 40 mg/kg administered dose when compared with the control. There was a slight decrease in the serum level of immunoglobulin G (IgG), for the dose of 60 mg/kg, though not statistically significant. However, a non-significant increase was observed for the dose of 80mg/kg administered when compared with the control. The serum level of immunoglobulin G (IgG) was also observed to be unchanged for the dose of 20mg/kg when compared with the control.

A significant increase in the serum level of immunoglobulin M (IgM) was seen following the administration of 40mg/kg of the extract when compared with the control. However, a non–significant increase in the serum level of immunoglobulin M (IgM) was recorded for the doses of 60mg/kg and 80mg/kg when compared with the control.

Table 1: Temperature, Weight and Other Observable Changes in the Wistar Rats after Treatment

Groups	Temperature (^{0}C)		Weight (g)		Other Observable after Experiment
	Before Experiment Mean±S.D	After Experiment Mean±S.D	Before Experiment Mean±S.D	After Experiment Mean±S.D	
A	37.2±1.00	37.9±1.00	180.0±20.0	180.0±20.0	None
B	37.1±1.00	41.2±2.00	180.0±20.0	120.0±20.0	Withdrawal behaviour, hair loss, sore on the legs
C	37.3±1.00	38.0±1.00	180.0±20.0	178.0±20.0	Same as control
D	37.0±1.00	37.1±1.00	180.0±20.0	185.0±20.0	Same as control
E	37.3±1.00	37.2±1.00	180.0±20.0	190.0±20.0	Same as control

Key:
SD: Standard Deviation
^{0}C: Degree Celsius
g: Grams
mg/kgbw – milligram/kilogram body weight

Table 2: Mean Serum Levels Of Immunoglobulins Following Administration of *Moringa Oleifera* Leaf Extract To Wistar Rats

Groups	**Treatment**	**Parameters**			**F-value**	**Experiment P-value**
		IgA (g/L) Mean ± SEM	**IgG (g/L) Mean ± SEM**	**IgM (g/L) Mean ± SEM**		
Group A	Control	0.25±0.16	3.25±0.16	0.75±0.16	0.895	0.65
Group B	20mg/kg	0.25±0.16	3.25±0.16	0.75±0.16	0.655	0.15
Group C	40mg/kg	1.50±0.27*	2.75±0.16*	1.38±0.32*	0.250	0.04
Group D	60mg/kg	2.00±0.78*	3.00±0.00	0.88±0.23	0.200	0.03
Group E	80mg/kg	0.57±0.20	3.71±0.29	0.86±0.14	0.542	0.23

Key:
SEM: Standard Error of Mean; Sample size (n) =5;
***:** Significant at P-value less than 0.05; **g/L:** Gram per Liter;
mg/kgbw – milligram/kilogram body weight
IgA: Immunoglobulin A; **IgG**: Immunoglobulin G
IgM: Immunglobulin M

4. Discussion

The effect of *Moringa oleifera* leave extract on the immunoglobulins of Wistar rats was investigated in this study. The assessment of immune parameters such as immunoglobulins is a biomarker for evaluating immune function and autoimmune conditions.

The results in Table 2 showed that there was a significant ($p<0.05$) increase in the serum level of immunoglobulin A (IgA) following the administration of 40mg/kg and 60mg/kg of the extract when compared with the control. The level of immunoglobulins A and M (IgA and IgM) in the rats administered with the *Moringa oleifera* leaf extract suggested that the leaf extract markedly enhanced the production of (IgA and IgM) and has immune modulatory activities. This study is in agreement with the study carried out by Smith (2008) and Patel *et al.*(2010).

Similarly, Table 2 also showed a significant increase in the serum level of immunoglobulin M (IgM) following the administration of 40mg/kg of the extract when compared with the control. However, a non–significant increase in the serum level of immunoglobulin M (IgM) was recorded for the doses of 60mg/kg and 80mg/kg when compared with the control. This result was also in agreement with Mahajan and Mehta (2009); Patel *et al.* (2010). Patel *et al.*(2010) reported that antibody molecules, a product of B-lymphocytes and plasma cells, are central to humoral immune responses, and that IgG and IgM are the major immunoglobulins which are involved in the complement activation, opsonization, neutralization of toxins.

Furthermore, Table 2 revealed a significant reduction in serum level of immunoglobulin G at the concentration of 40mg/kg of the extract when compared with the control. This study is in agreement with works done by Adedapo *et al.* (2010); Muchenje *et al.* (2012); Eme, (2013). In their studies, Adedapo *et al.*(2010) reported that higher dose of the extract showed a significant dose dependent high serum levels of Immunoglobulin A. Eme, (2013) added that immunoglobulins are normally produced by B cell to regulate immune system especially humoral immunity, and their production is in response to environmental substances (molecules or microbes) that gain access into the body.

This study further revealed in Table 2 that there was a slight decrease in the serum level of immunoglobulin G (IgG), for the dose of 60 mg/kg, though not statistically significant. However, a non-significant increase was observed for the dose of 80mg/kg administered when compared with the control. The serum level of immunoglobulin G (IgG) was also observed to be unchanged for the dose of 20mg/kg when compared. This study is in agreement with Nickon *et al.* (2003); Carrasco *et al.*(2009); Kasolo *et al.*(2011).

The findings of this research work suggest immunomodulatory potential of the extract of *Moringaoleifera*. This is also in agreement with Calis*et al.,* (1997) and Gupta *et al.,* (2010), who reported in their separate studies that extracts from *M. oleifera* leaves modulate humoral immunity in rats and mice. This present study showed that *Moringa oleifera* leaf extract has the potential to boost the capacity of the host to fight invading organisms. Furthermore, *Moringa oleifera* leaf extract may possess a possible beneficial therapeutic effect on the amelioration of immunological diseases such as systemic lupus erythematosus (SLE), rheumatoid arthritis and psoriasis in humans, as well as prevent *Staphylococcus* and *Streptococcus* infection. The overall trend obtained in the parameters employed for the assessment of immunomodulatory potentials of the leaf extract of *Moringa oleifera* indicated that the extract is a good substance as an immune modulating regime.

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