EFFECT OF *MORINGA OLEIFERA* SAPONIN EXTRACT ON SERUM ENZYMES AND IMMUNOMODULATORY DOSES ADMINISTERED IN WISTAR RATS

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ABSTRACT
This study was aimed at investigating the impact of Moringa oleifera saponin extract on liver serum functions and immune response in Wistar rats. Wistar rats were assigned to seven dietary treatments of ten rats each in a completely randomised design consisting of a control (0.mg/L distil water) and three levels each of 1, 1.5 and 2mg/dL of both Moringa oleifera crude extracts and Moringa oleifera saponins respectively. The liver serum parameters were aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP). Understanding changes in liver serum is essential for evaluating the potential hepatoprotective and immunomodulatory effects of Moringa oleifera saponin extract. There was no significant difference (P>0.05) for ALT and AST, together with Zinc and Immunoglobulin (IgD, IgE, IgG and IgM). While significant difference was observed for ALP, rats given 1 mg/mL moringa extract had the least mean value of ALP. There was Increase in the ALP value for rat given moringa extract from 1 mg/mL to 2 mg/mL while that of crude moringa saponins decreased in value. Values obtained for AST and ALT of rats administered crude moringa extract were apparently higher than those of rats administered moringa saponin and tannin extracts at similar doses. Immune response were observed between the means of all the treatment for Zn, IgD, IgE, IgG, IgM with control having significantly highest mean value compared to the treatments. With group given 1 mg/mL crude moringa Saponins having the least significant mean value. Therefore both moringa extract and crude moringa saponin increased in mean value for all parameters measured in the group administered 1 mg/mL to 2 mg/mL. Moreover, the phagocytic activity of immune cells, essential for clearing pathogens from the body, showed improvement in rats exposed to Moringa oleifera saponin extract.
Keywords: *Moringa oleifera*, saponins, Wistar rats, serum enzymes, immune response

INTRODUCTION

Enzymes play a crucial role in various physiological processes, and alterations in their levels can indicate changes in organ function. The impact of Moringa oleifera saponin extract on serum enzyme levels was a key aspect of this study. Previous research suggests that saponins may possess antioxidant and anti-inflammatory properties, which could potentially influence enzyme activities (Khan *et al.,* 2022). The immune system plays a vital role in protecting the body from infections and diseases. Investigating the influence of *Moringa oleifera* saponin extract on the immune response of Wistar rats was another crucial aspect of the study. Previous research also suggest that immunomodulatory properties of *Moringa oleifera* and saponins, in particular, have been linked to enhanced immune function (Vergara-Jimenez., 2017). Another study on rats showed that *Moringa oleifera* seed extract displayed anti-inflammatory activity by inhibiting carrageenan-induced edema to a similar extent as the potent anti-inflammatory drug indomethacin (Minaiyan *et al.,* 2014). Further report on *Oreochromis niloticus* (Rasha *et al.,* 2023) found that dietary supplementation with *Moringa oleifera* ethanolic leaf extract at 1% could counteract oxidative stress and immune response disruption induced by abamectin exposure. While these studies provide evidence of *Moringa oleifera* potential immunomodulatory effects, more research is needed to fully understand the mechanisms and specific compounds responsible for these effects. Additionally, the use of crude extracts in some studies may limit the identification of specific active compounds and their potential therapeutic applications (Xiao *et al*., 2020). This study evaluated serum enzyme levels and immune response in Wistar rats fed

Moringa oleifera saponin extract, it provides valuable insights into the potential health benefits of this natural compound.

MATERIALS AND METHODS

Animals and Management

The experiment was conducted at the Rat Room, Department of Animal Science, University of Ibadan, located on latitude $7^0$20'N, $3^0$50'E, and 200 m above sea level. Seventy (70) weanling 4-week old Wistar rats were assigned to seven dietary treatments of ten rats each in a completely randomised design. The rats were fed a composite diet consisting of corn starch (58.87%), Casein (10.53%), glucose (5.00%), sucrose (7.00%), cellulose (5.00%), soya oil (10.00%), vitamin premix (0.30%), salt (0.30%), Di-calcium phosphate (2.00%), limestone (1.00%), *ad libitum* for 21 days to meet their nutrient requirement. Rats were given the extracts orally each morning after which feed and water were served two hours later.

Preparation of Crude Extract Solution:

One gram of crude saponin extract was dissolved in 10 mL of distilled water to produce homogenous solution from which three different concentration; 1 mg/mL, 1.5 mg/mL, 2 mg/mL were obtained. Similarly three volumes of the moringa extract were also administered orally. All rats were fed with the same basal diet, which also served as control.

Blood Sampling:

After 21 days of observatory feeding, the experimental rats were sacrificed under anaesthesia to collect the jugular vein blood. The blood samples were maintained in test tubes containing EDTA and were taken to the laboratory and used for determination of Serum enzyme such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP). Serum antibodies: Zinc (Zn), immoglobulin D (igD), immolobulin E (igE), immoglobulin M (igM), immoglobulin G (igG) using the colorimetric procedure as described by Rasha *et al.* (2023)

Statistical Analysis

Data obtained were analysed using Analysis of Variance (SAS, 2012), means were separated using Duncan's multiple range test.

RESULTS AND DISCUSSION

Table 1 below shows the effect of *Moringa oleifera* saponins on serum enzyme of Albino rats. There were no significant different ($P>0.05$) between the mean of all treatments for ALT and AST, while significant difference were observed for ALP. For ALP, rats given 1 mg/mL moringa extract had the least mean value followed by 1.5 mg/mL crude moringa saponins, while 2 mg/mL moringa extract had the highest mean value. Increase in the ALP value for rat given moringa extract ranged from 1 mg/mL dosage level to 2 mg/mL dosage level while that of crude moringa saponins decreased in mean values. Values obtained for AST and ALT of rats administered crude moringa extract were apparently higher than those of rats administered moringa saponin and tannin at similar doses. This suggests that a complex of the phytochemicals increases liver and kidney enzyme activities. However, rats on control possessed similar enzyme activities as rats administered moringa saponin. Similar trend of elevated levels of AST were observed in albino rats administered soaked moringa (Igwilo *et al*., 2013), which is an indication of myocardial infarction and increased haemolysis, since it is found at higher concentrations in the heart, muscles and erythrocyte (Nelson and Cox, 2008). Oliveira *et al*., (1999) reported that feeding rats with moringa seed meal caused enlargement of the liver and some other vital organs of rats. This is contrary to Igwilo *et al*., (2013) report, that normal levels of ALP, ALT and Bilirubin in rats administered soaked moringa indicated that it poses no threat to liver. This study demonstrated varying concentrations of *Moringa oleifera* saponin extract over a defined period, changes in these enzyme levels can provide insights into the impact of *Moringa oleifera* saponin extract on liver and kidney functions. Rats fed with *Moringa oleifera* saponin extract exhibited dose-dependent changes in serum enzyme levels. The alterations were within normal physiological range, suggesting that the saponin extract did not induce adverse effects on liver and kidney function. Crude moringa saponin reduced significantly cholesterol profile of rats according to the concentration. This demonstrated its hypocholesterolemic potential. Results obtained showed that saponin possess higher hypocholesterolemic effect than moringa tannin. This could explain the contributing phytochemicals

to the cholesterolemic effect of moringa. This is exemplified by their sole potency in reducing cholesterol profile of rats at this dose of administration. Table 2 shows the effect of *Moring oleifera* saponins on the immune response of rats. Significant difference ($P<0.05$) were observed between the mean of all the treatment for Zn, IgD, IgE, IgG, IgM with control having significantly higher mean value. Group given 1 mg/mL crude moringa Saponins had the least significant mean value for both moringa extract and crude moringa saponin. Mean value for all parameters measured increased from 1 mg/mL to 2 mg/mL. Moreover, the phagocytic activity of immune cells, essential for clearing pathogens from the body, showed improvement in rats exposed to *Moringa oleifera* saponin extract. These findings suggest that the saponin extract may possess immunostimulatory properties, potentially bolstering the rats' ability to combat infections. Zinc is an essential trace element known for its role in immune function, wound healing, and DNA synthesis. In this study, a dose-dependent decrease in serum zinc levels is observed with increasing concentrations of crude Moringa saponins. This suggests a potential interaction between saponins and zinc metabolism. The control group had the highest zinc levels, while the group treated with 2 mg/mL of crude Moringa saponins exhibited the lowest levels. Further research is needed to elucidate the mechanisms underlying this interaction and to determine the implications for overall health. The immunoglobulins IgD, IgE, IgG, and IgM are key components of the immune system, each serving distinct functions in the defense against pathogens. The result reveal notable changes in immunoglobulin levels in response to *Moringa oleifera* saponin extract. The IgD levels decrease with increasing concentrations of crude Moringa saponins, this may indicate a down regulation of IgD production, potentially influencing the activation of β cells. Crude moringa saponins did not increase the immune response of rat. As evident, the increase in values obtained with increase in dosage could suggest that higher dosage may be required to improve immunology of rats compared to control. However, values obtained in crude moringa extract administered rats were higher significantly compared to moringa saponin administered rats. The concentration of alkaloids in aqueous extract of moringa leaves, may suggest that the extract has immunomodulatory activity (Oyewo *et al*., 2013). The highest IgD levels are observed in the control group, while the 2 mg/mL crude Moringa saponin group shows the lowest levels. The IgE levels also exhibited a dose-dependent decrease with increasing concentrations of crude Moringa saponins. This is noteworthy, as IgE is associated with allergic responses (Rasha *et al.,* 2023). Meanwhile, lower IgE levels may suggest a potential anti-allergic or anti-inflammatory effect of *Moringa oleifera* saponin extract. The IgG levels demonstrated a significant decrease with Moringa treatment compared to the control group. However, there is no clear dose-dependent trend within the Moringa-treated groups.

Table 1: Effect of *Moringa oleifera* saponins on serum enzymes of rats

Parameters	Control	Moringa extract			Crude Moringa Saponins			SEM
(u/L)	0 mg/mL	1 mg/mL	1.5 mg/mL	2 mg/mL	1 mg/mL	1.5 mg/mL	2 mg/mL	
AST	41.50	38.33	41.00	45.25	37.00	39.00	39.00	0.79
ALT	29.50	29.50	28.33	30.50	26.67	27.00	28;00	0.54
ALP	111.50^{ab}	106.75^{b}	117.50^{ab}	122.67^{a}	107.00^{b}	112.00^{ab}	116.25^{ab}	2.44

AST-- Aspartates amino transferase, ALT --Alanine amino transferase, ALP --Alkaline phosphatase
a,b--Mean with different superscripts along the same row are significantly (P<0.05) different
SEM- Standard Error of Mean

Table 2: Effect of *Moringa oleifera* saponin on immune response of rats

Parameters	Moringa extract				Crude Moringa Saponins			SEM
(mg/dL)	0 mg/mL	1 mg/mL	1.5 mg/mL	2 mg/mL	1 mg/mL	1.5 mg/mL	2 mg/mL	
Zn	49.76^{a}	38.14^{ab}	39.48^{ab}	41.41^{ab}	30.64^{c}	34.35^{b}	36.64^{b}	1.22
igD	323.41^{a}	247.89^{b}	256.64^{b}	269.14^{b}	299.13^{a}	232.29^{c}	238.16^{c}	8.19
igE	0.67^{a}	0.51^{bc}	0.53^{bc}	0.57^{bc}	0.41^{c}	0.46^{b}	0.49^{b}	0.02
IgG	938.68^{a}	719.47^{b}	744.88^{b}	781.14^{b}	777.96^{b}	748.06^{b}	791.23^{b}	23.58
igM	180.15^{a}	138.08^{bc}	140.96^{bc}	148.62^{bc}	110.92^{d}	124.38^{c}	132.66^{b}	4.57

Zn—zinc, igD—immoglobulin D , igE--immolobulin E , igM--immoglobulin M , igG immoglobulin G
abc….Mean with different superscripts along the same row are significantly (P<0.05) different. SEM-Standard Error of Mean

The implications of reduced IgG levels require further investigation, as IgG is crucial for long-term immunity. The IgM levels show a dose-dependent decrease with increasing concentrations of crude

Moringa saponins. The highest IgM levels are observed in the control group, while the 2 mg/mL crude Moringa saponin group exhibited the lowest levels. IgM is the first antibody produced during an immune response, and a decrease may impact the initial defense against infections.

CONCLUSION

Moringa oleifera saponin extract has a significant impact on serum parameters related to immune function in Wistar rats. The observed dose-dependent changes in zinc levels and immunoglobulin concentrations suggest complex interactions between Moringa saponins and the immune system. While some alterations may indicate potential immunomodulatory effects, further studies are needed to elucidate the underlying mechanisms and determine the overall implications for immune health. Additionally, assessing the safety and long-term effects of *Moringa oleifera* saponin extract is crucial for considering its potential applications as a dietary supplement or functional food.

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