

Research Article**Effect of methanol extract of *Moringa oleifera* Lam (Moringaceae) Young Pods on some Serum biochemical parameters in wistar rats**H. A. Madziga^{1*}, O. A. Sodipo², J. G. Usman³, D. Yah¹, M. Chiroma¹, N. A. Ojo¹¹Department of Veterinary Physiology, Pharmacology and Biochemistry, University of Maiduguri, Borno State, Nigeria.²Department of Clinical Pharmacology and Therapeutics, College of Medical Sciences, University of Maiduguri, Maiduguri, Borno State, Nigeria.³National Veterinary Research Institute Vom, P.M.B. 01, Plateau State, Nigeria

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Abstract

Objective: to study the effects of methanol extract of young pods of *Moringa oleifera* on some biochemical parameters in wistar rats. **Materials and methods:** A total of 80 apparently healthy rats of both sexes were used for this experiment and were randomly divided into 4 groups (A, B, C, D) of 20 rats each. Group A served as the control and received distilled water throughout the experimental days; while groups B, C and D were given methanol extract of young pods of *Moringa oleifera* at a dose range of 200, 400 and 600 mg/kg body weight respectively for 21 days. The following serum biochemical parameters were observed at the end of every week (7 days), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP), total protein, total albumin, total cholesterol, urea, creatinine and electrolytes (Sodium, Potassium, Calcium and Inorganic Phosphate).

Results and conclusion: The result of the study on serum albumin showed that the extract on initial administration caused a significant ($P<0.05$) decrease of 27.60 ± 1.14 mg/L, 18.00 ± 0.71 mg/L and 23.40 ± 1.14 mg/L respectively when compared to 39.00 ± 0.71 mg/L of control at day 7 of treatment. Day 14 showed a significant ($P<0.05$) increase compared to 26.20 ± 0.84 mg/L of control while day 21 indicated no change. Cholesterol level at day 7 showed a significant ($P<0.05$) decrease of 1.26 ± 0.11 mg/L, 0.74 ± 0.11 mg/L and 0.60 ± 0.07 mg/L respectively compared to control of 1.50 ± 0.07 mg/L. Sodium level at day 7 with extract doses of 200 mg/kg, 400 mg/kg and 600 mg/kg showed a significant ($P<0.05$) decrease of 110.00 ± 0.71 mg/L, 70.20 ± 0.84 mg/L and 100.40 ± 0.55 mg/L respectively compared to 135.60 ± 0.55 control. Potassium at day 7 with 400 mg/kg dose showed a significant ($P<0.05$) decrease of 2.50 ± 0.07 mg/L. There were significant ($P<0.05$) decreases in the levels of serum enzymes, AST, ALT and ALP. Therefore it was concluded that prolonged administration of methanolic extract of young pods of *Moringa oleifera*, increased serum albumin, decrease serum cholesterol, decrease serum sodium, increase potassium and calcium, decrease urea and creatinine levels and caused decrease in AST, ALT and ALP enzymes.

Keywords: Methanol extract, young pods, *Moringa oleifera*, serum biochemicals

Introduction

In Nigeria, herbal medicine has become part of the peoples' culture with about half of the population relying on traditional medicine (Geidam et al., 2007), preparations from plants have given hope to many in the quest for treating old and emerging diseases that have defied many orthodox drugs, hence, the need to incorporate traditional medicine to our modern health care services. Plant medicines are known to be safe and better for

human health than the synthetic drugs (Kilham, 1999). One of such plants is *Moringa oleifera* in which indigenous people have found much success in using various parts of the tree to cure many diseases and physical ailments (Fahey, 2005). The plant is known to be a natural antihelmintic, antibiotic, detoxifier and immune booster and is used in many countries to treat malnutrition and malaria (Ramachandran et al., 1980). Also, the juice from the leaves is believed to stabilize blood pressure, the flowers are used to cure inflammations, the pulps are used for joint pain, the roots are used to treat rheumatism and the bark can be chewed to aid in digestion (Ram, 1994).

Tissue damage resulting from injury or disease is generally accompanied by increase in the levels of several non-

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functional enzymes (Murray et al., 2003). For example, increased serum aspartate aminotransferase (AST) may be diagnostic of liver damage or myocardial infarction or viral hepatitis. Similarly, increased serum alkaline phosphatase (ALP) may be indicative of obstructive liver disease or various bone disorders (Murray et al., 2003). Other serum biochemical findings that are reflective of liver damage include hyperbilirubinaemia (unconjugated), hypoproteinaemia (albumins, globulins and clotting (Cotron et al., 1999). Major serum biochemical changes associated with severe renal damage include increased blood-urea-nitrogen (BUN) and serum creatinine values (azotemia), hypoproteinaemia (involving usually the albumin fraction), hyperkalemia, hyperphosphatemia and hypocalcaemia (Cotron et al., 1999).

This study aims at investigating the effects of methanol extract of *Moringa oleifera* young pod on some biochemical parameters in Wistar albino rats.

Materials and methods

Plant identification, collection and extract preparation

Fresh *Moringa oleifera* young pods were collected from a natural habitat in Mairi village, Jere Local Government Area (LGA) of Borno state, Nigeria, in January 2014. The plant materials were identified by a taxonomist from the Department of Biological Sciences, University of Maiduguri. The young pods were cut into small pieces and air dried at room temperature. The crushing of the young pods was done using a mortar and pestle, after which it was grounded into powder. Two hundred and ninety grams (290 g) of the powder was weighed and introduced into a conical flask and 1 litre of Methanol was added thereafter. The mixture was then shaken and allowed to stand for 30 minutes; this was steamed for one hour and was allowed to cool before filtering using whatman No. 1 filter paper. The filtrate was oven dried at 40°C for 24 hours and thereafter stored in a glass container at 4°C in a refrigerator until required.

Experimental animals

A total of apparently healthy 80 albino Wistar rats of both sexes weighing between 145 and 200g were used for the experiments which were obtained from the animal house, Department of Veterinary Physiology and Biochemistry, University of Maiduguri. The rats were kept in plastic cages and allowed to adjust to the laboratory environment for a period of two weeks and screened for some infectious diseases before the commencement of the experiments. They were fed with growers mash (Vital Feed Nig. Ltd.) and water was provided *ad libitum*. All experiments on animals were carried out according to the biomedical principles involving animals (CIOMS and ICLAS, 2012).

The rats were divided at random into four groups A, B, C and D of twenty rats each. Group A served as the control and the rats were given distilled water, while groups B, C and D were given the extract orally at doses 200 mg/kg, 400 mg/kg, and 600 mg/kg respectively for three consecutive weeks. Blood samples collected were centrifuged at 1200 rpm for 5min and serum harvested for determination of serum biochemical analyses such as (Total protein, Albumin, Cholesterol, Urea, Creatinine) as describe by different researchers (Peter et al., 1982; Sood, 2006; Allain et al., 1974; Kaplan and Pesche, 1989; Seaton and Ali, 1984). The electrolytes (Sodium, Potassium, Calcium, Inorganic phosphates) were determined by the method described by Tietz (2006). The enzymes AST, ALT and ALP (aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase respectively) were determined by Reithman and Frankel method 1957 and the method of Tietz 2006.

Statistical Analysis

The data collected were presented as mean \pm standard deviation (S.D.). One way analysis of variance (ANOVA) was used to analyze the differences among the means. The value of $P < 0.05$ was considered significant. Computer statistical software the GraphPad Instat[®] (2003) was used.

Results

Effect of methanol extract of Young Pods of *Moringa oleifera* on serum Protein, Albumin and Cholesterol in albino rats

The effects of prolonged oral administration of the extract of young pods of *Moringa oleifera* on total protein, serum albumin and cholesterol levels are presented in table 1.

At days 7, 14 and 21 of treatment with 200 mg/kg, 400 mg/kg and 600 mg/kg doses respectively, protein values indicated a significant ($P < 0.05$) decrease for all the doses when compared with their controls, while at day 7 of extract withdrawal a significant ($P < 0.05$) decrease was only recorded at 400mg/kg.

Serum albumin level at day 7 indicated a significant ($P < 0.05$) decrease of 27.60 ± 1.14 mg/L, 18.00 ± 0.71 mg/L and 23.40 ± 1.14 mg/L with doses 200 mg/kg, 400 mg/kg and 600 mg/kg respectively when compared to 39.00 ± 0.71 mg/L of control. Day 14 showed a significant ($P < 0.05$) increase of 34.20 ± 0.84 mg/L, 36.20 ± 0.84 mg/L and 37.00 ± 0.71 mg/L with doses 200 mg/kg, 400 mg/kg and 600 mg/kg respectively when compared to 26.20 ± 0.84 mg/L of control. At day 21 extract doses of 200mg/kg and 600 mg/kg indicated a significant ($P < 0.05$) decrease of 35.20 ± 0.84 mg/L and 29.40 ± 1.14

Table 1. Effects of prolonged oral administration of methanol extract of Young Pods of *Moringa oleifera* on some biochemical parameters in wistar rats

Parameter	Dose of Extract (mg/kg)	Days of Treatment			Days of Extract withdrawal
		7	14	21	7
Total protein (g/L)	Control (Distilled water)	80.40±1.14	51.80±4.44	79.00±0.71	71.40±1.14
	200	52.20±0.84 ^b	42.00±1.00 ^b	72.20±0.84 ^b	70.40±1.14
	400	28.00±0.71 ^b	67.40±1.14 ^a	79.20±0.84	62.80±1.48 ^b
	600	42.00±0.71 ^b	41.40±1.14 ^b	76.40±1.14 ^b	72.80±1.48
Serum Albumin (g/L)	Control(Distilled water)	39.00±0.71	26.20±0.84	37.70±0.71	37.20±0.84
	200	27.60±1.14 ^b	34.20±0.84 ^a	35.20±0.84 ^b	33.20±0.84 ^b
	400	18.00±0.71 ^b	36.20±0.84 ^a	37.20±0.84	31.40±1.14 ^b
	600	23.40±1.14 ^b	37.00±0.71 ^a	29.40±1.14 ^b	38.40±1.14 ^a
Total Cholesterol (mg/L)	Control(Distilled water)	1.50±0.07	1.20±0.08	1.40±0.07	1.52±0.15
	200	1.26±0.11	1.50±0.07 ^a	1.82±0.08 ^a	2.14±0.11 ^a
	400	0.74±0.11 ^b	1.44±0.11 ^a	1.54±0.11	1.38±0.15
	600	0.60±0.07 ^b	1.32±0.08 ^a	1.24±0.11	1.56±0.11

a = Significant (P<0.05) increase as compared to control; b = Significant (P<0.05) decrease as compared to control (n = 5)

mg/L respectively as compared to 37.70±0.71 mg/L control. The 400 mg/kg dose showed no significant (P>0.05) change as compared to control. At day 7 of extract withdrawal, 200 mg/kg and 400 mg/kg showed a significant (P<0.05) decrease while dose 600 mg/kg indicated a significant (P>0.05) increase compared to control.

Total cholesterol at day 7 indicated significant (P<0.05) decrease of 1.26±0.11 mg/L, 0.74±0.11 mg/L and 0.60±0.07 mg/L at doses 200 mg/kg, 400 mg/kg and 600 mg/kg respectively as compared to 1.50±0.07mg/L control. Day 14 indicated a significant (P<0.05) increase of 1.50±0.07 mg/L, 1.44±0.11 mg/L and 1.32±0.08 mg/L compared to 1.20±0.08 mg/L of control. Day 21 of extract treatment showed no significant (P>0.05) change as compared to control except at dose 200mg/kg which showed a significant (P<0.05) increase of 1.82±0.08 mg/L compared to 1.40±0.07 mg/L of control. Day 7 of extract withdrawal indicated showed a significant (P<0.05) increase of 2.14±0.11 mg/L as compared to 1.52±0.15 mg/L of control with the 200mg/kg dosage.

Effect of prolonged oral administration of methanol extract of Young Pods of *Moringa oleifera* on Serum Electrolytes

The results of the effect of prolonged oral administration of the extract on sodium, potassium, calcium and Inorganic phosphate are presented in table 2

Sodium level at day 7 of treatment indicated a significant

(P<0.05) decrease of 110.00±0.71 mg/L, 70.20±0.84 mg/L and 100.40±0.55 mg/L with doses 200 mg/kg, 400 mg/kg and 600 mg/kg respectively compared to 135.60±0.55 mg/L of control. Day 14 showed a significant (P<0.05) increase of 120.80±0.84 mg/L, 141.00±0.71 mg/L and 141.20±1.30 mg/L with doses 200 mg/kg, 400 mg/kg and 600 mg/kg respectively compared to 110.00±0.71 mg/L of control. Day 21 showed a significant (P>0.05) increase with doses dose 400 mg/kg and 600 mg/kg. Dose 200 mg/kg showed no significant (P<0.05) change compared to the control. At day 7 of extract withdrawal, there was a significant (P<0.05) decrease with dose 600 mg/kg.

Potassium level at day 7 of treatment with 200 mg/kg, 400 mg/kg and 600 mg/kg doses of the extract indicated a significant (P<0.05) decrease of 4.46±0.05 mg/L, 2.50±0.07 mg/L and 3.60±0.07 mg/L respectively when compared to 5.26±0.05 mg/L of control. At day 14, treatment with the same dose indicated a significant (P<0.05) increase of 5.36±0.09 mg/L, 6.28±0.08 mg/L and 6.40±0.10 mg/L respectively compared to 3.80±0.07 mg/L of control. Day 21 treatment with 400 mg/kg showed a significant (P<0.05) increase of 7.16±0.89 compared to 6.30±0.07 mg/L control while treatment with 200 mg/kg and 600 mg/kg showed no significant (P>0.05) change.

At day 7 of extract withdrawal significant (P<0.05) increase at 400 mg/kg dose of the extract was seen compared to

Table 2. Effect of Prolonged oral Administration of Methanol Extract of Young Pods of *Moringa oleifera* on Serum Electrolytes

Parameters	Dose of extract (mg/kg)	Days of treatment (Mean± SD)			Days of extract withdrawal
		7	14	21	7
Sodium	Control (Distilled water)	135.60±0.55	110.00±0.71	140.00±0.71	140.40±1.14
	200	110.00±0.71 ^b	120.80±0.84 ^a	140.80±0.84	140.40±1.14
	400	70.20±0.84 ^b	141.00±0.71 ^a	144.60±0.89 ^a	140.60±1.52
	600	100.40±0.55 ^b	141.20±1.30 ^a	141.00±1.41 ^a	136.00±2.12 ^b
Potassium	Control (Distilled water)	5.26±0.05	3.80±0.07	6.30±0.07	5.46±0.11
	200	4.46±0.05 ^b	5.36±0.09 ^a	6.54±0.11	5.82±0.15
	400	2.50±0.07 ^b	6.28±0.08 ^a	7.16±0.89 ^a	6.08±0.16 ^a
	600	3.60±0.07 ^b	6.40±0.10 ^a	6.40±0.10	5.06±0.15
Calcium	Control (Distilled water)	2.10±0.07	1.80±0.07	2.60±0.07	2.48±0.15
	200	1.58±0.08 ^b	2.40±0.10 ^a	2.62±0.08	2.48±0.08
	400	1.42±0.08 ^b	2.70±0.10 ^a	2.64±0.11	2.54±0.11
	600	1.20±0.07 ^b	2.72±0.13 ^a	2.62±0.13	2.58±0.08
Inorganic Phosphate	Control (Distilled water)	3.70±0.07	2.20±0.07	3.86±0.05	3.76±0.11
	200	2.80±0.07 ^b	3.22±0.13 ^a	3.48±0.08	3.32±0.13
	400	1.30±0.07 ^b	3.68±0.08 ^a	4.28±0.08 ^a	2.64±0.11 ^b
	600	2.56±0.09 ^b	3.30±0.10 ^a	4.08±0.08 ^a	3.48±0.15

a = Significant (P<0.05) increase as compared to control along the same Column; b = Significant (P<0.05) decrease as compared to control along the same Column (n=5)

Table 3. Effects of Prolonged Oral Administration of Methanol Extract of Young Pods of *Moringa oleifera* on Serum Urea (µmol/L) and Creatinine (µmol/L) levels in wistar rats

Parameters	Dose of Extract (mg/kg)	Days of treatment Mean± SD			Days of extract withdrawal
		7	14	21	7
Urea (µmol/L)	Control (Distilled water)	3.10±0.07	2.42±0.08	6.46±0.09	2.66±0.13
	200	2.74±0.11 ^b	4.66±0.09 ^a	6.12±0.13	5.88±0.08 ^a
	400	2.46±0.09 ^b	3.28±0.08 ^a	3.12±0.13 ^b	2.80±0.12
	600	3.06±0.09	2.70±0.07	3.58±0.08 ^b	3.08±0.16 ^a
Creatinine (µmol/L)	Control (Distilled water)	48.00±0.71	42.00±0.71	82.00±0.71 ^a	67.20±1.48
	200	38.40±0.55 ^b	71.60±0.89 ^a	71.00±1.00 ^b	160.80±0.84 ^a
	400	18.60±0.89 ^b	76.20±0.84 ^a	66.80±0.84 ^b	71.60±1.14 ^a
	600	21.60±0.89 ^b	78.40±0.55 ^a	59.20±0.84 ^b	46.40±1.14 ^b

a = significant (P<0.05) increase as compared to control along the same column; b = significant (P<0.05) decrease as compared to control along the same column; n = 5

control.

Calcium level at day 7 of treatment with doses 200 mg/kg, 400 mg/kg and 600 mg/kg indicated a significant (P<0.05) decrease of 1.58±0.08 mg/L, 1.42±0.08 mg/L and 1.20±0.07 mg/L respectively compared to 2.10±0.07 mg/L of control. Day 14 showed a significant (P<0.05) increase of 2.40±0.10 mg/L, 2.70±0.10 mg/L and 2.72±0.13 mg/L with doses 200 mg/kg, 400 mg/kg and 600 mg/kg respectively as compared to 1.80±0.07 mg/L of control. Day 21 of treatment showed no significant (P>0.05) changes compared to control. Day 7 of extract withdrawal also indicated no significant (P>0.05) change.

Inorganic phosphate level at day 7 of extract treatment with doses

200 mg/kg, 400 mg/kg and 600 mg/kg indicated a significant (P<0.05) decrease of 2.80±0.07 mg/L, 1.30±0.07 mg/L and 2.56±0.09 mg/L respectively when compared to 3.70±0.07 mg/L of control. Days 14 and 21 indicated significant (P<0.05) increases compared to control. But there was no significant (P>0.05) change at day 21 with 200 mg/kg dose. At day 7 of extract withdrawal, there was a significant (P<0.05) decrease in phosphate level of 2.64±0.11 mg/L compared to 3.76±0.11 of control at dose 400 mg/kg.

Effects of prolonged oral administration of methanol extract of Young Pods of *Moringa oleifera* on Serum

Table 4. Effects of prolonged oral administration of methanol extract of Young Pods of *Moringa oleifera* on enzymes in wistar rats

Parameter/units	Dose of extract (mg/kg)	Days of treatment (Mean± SD)			Days of extract withdrawal
		7	14	21	7
Aspartate Aminotransferase (AST) IU/L	Control (Distilled water)	36.00±0.71	47.40±1.14	89.60±0.89	47.40±1.14
	200	26.60±0.89 ^b	42.00±1.00 ^b	89.20±0.84	104.60±1.14 ^a
	400	10.80±0.84 ^b	67.40±1.14 ^a	98.60±1.14 ^a	67.20±1.48 ^a
	600	16.40±1.14 ^b	41.40±1.14 ^b	67.40±1.14 ^b	58.40±1.14 ^a
Alanine Aminotransferase (ALT) IU/L	Control (Distilled water)	17.00±0.71	18.60±0.89	25.80±0.84	29.40±1.14
	200	12.60±0.89 ^b	12.60±0.89 ^b	12.60±0.89 ^b	38.80±0.84 ^a
	400	4.60±0.89 ^b	17.60±0.89 ^b	25.60±0.89	38.60±1.14 ^a
	600	8.60±0.89 ^b	16.40±1.14 ^b	12.20±0.84 ^b	26.20±1.30 ^b
Alkaline Phosphatase (ALP) IU/L	Control (Distilled water)	160.40±1.14	300.00±1.00	453.60±1.14	392.40±1.52
	200	87.40±1.14 ^b	159.60±1.52 ^b	88.40±1.14 ^b	396.40±1.34
	400	52.80±0.84 ^b	169.20±0.84 ^b	204.60±1.14 ^b	249.00±0.71 ^b
	600	31.80±0.84 ^b	226.60±1.14 ^b	151.00±0.71 ^b	483.60±2.51 ^a

a = significant (P<0.05) increase as compared to control along the same column; b = significant (P<0.05) decrease as compared to control along the same column; n = 5

Urea (µmol/L) and Creatinine (µmol/L) levels in wistar rats

The result of the effect of prolonged oral administration of methanol extract of *Moringa oleifera* young pods on urea and creatinine are presented in table 3.

Urea level at day 7 of treatment indicated a significant (P<0.05) decrease of 2.74±0.11 µmol/L and 2.46±0.09 µmol/L with doses 200 mg/kg and 400mg/kg respectively when compared to 3.10±0.07 µmol/L of control, but at 600 mg/kg extract dose, there was no significant (P>0.05) change compared to control. At day 14, administration of 200 mg/kg and 400mg/kg extract dose showed a significant (P<0.05) increase of 4.66±0.09 µmol/L and 3.28±0.08 µmol/L respectively compared to 2.42±0.08 µmol/L of control, while at 600 mg/kg there was no significant (P>0.05) change as compared to the control group. At day 21, treatment with 200 mg/kg extract dose showed no significant change compared to control group, but at 400 mg/kg and 600 mg/kg extract dose a significant (P<0.05) decrease of 3.12±0.13 µmol/L and 3.58±0.08 µmol/L respectively were shown compared to 6.46±0.09 µmol/L of control. At day 7 of extract withdrawal, a significant (P<0.05) increase of 5.88±0.08 µmol/L and 3.08±0.16 µmol/L with 200 mg/kg and 600mg/kg respectively were observed compared to 2.66±0.13 µmol/L of control.

Creatinine level at day 7 of treatment with 200 mg/kg, 400 mg/kg and 600 mg/kg doses of the extract indicated a significant (P<0.05) decrease of 38.40±0.55 µmol/L, 18.60±0.89 µmol/L and 21.60±0.89 µmol/L respectively compared to 48.00±0.71 µmol/L of control. Day 14 of treatment using the same doses showed a significant (P<0.05) increase of 71.60±0.89 µmol/L,

76.20±0.84 µmol/L and 78.40±0.55 µmol/L respectively as compared to 42.00±0.71 µmol/L of control. Day 21 of treatment indicated a significant (P<0.05) decrease of 71.00±1.00 µmol/L, 66.80±0.84 µmol/L and 59.20±0.84 µmol/L respectively compared to 82.00±0.71 µmol/L of control. At day 7 of extract withdrawal, there was a significant (P<0.05) increase with doses 200 mg/kg and 400 mg/kg, but at 600 mg/kg there was a significant (P<0.05) decrease compared to the control group.

Effects of prolonged oral administration of methanol extract of Young Pods of *Moringa oleifera* on Serum Enzymes in wistar rats

The effect of prolonged oral administration of methanol extract of young pods of *Moringa oleifera* on aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) is presented in table 4.

AST levels at day 7 showed a significant (P<0.05) decrease of 26.60±0.89 iu/L, 10.80±0.89 iu/L and 16.40±1.14 iu/L with doses 200 mg/kg, 400 mg/kg and 600 mg/kg respectively compared to 36.00±0.71 iu/L of control. Day 14 showed a significant (P<0.05) decrease in AST levels of 42.00±1.00 iu/L and 41.40±1.14 iu/l with doses 200 mg/kg and 600 mg/kg respectively. At day 21, there was a significant (P<0.05) increase in AST level of 98.60±1.14 iu/L with dose 400 mg/kg compared with the control (89.60±0.89 iu/L), while with extract dose of 600 mg/kg, there was a significant (P<0.05) decrease (67.40±1.14 iu/L) in AST level. At day 7 of extract withdrawal, there was a

significant ($P<0.05$) increase in AST level compared to control.

ALT level at day 7 indicated a significant ($P<0.05$) decrease of 12.60 ± 0.89 iu/L, 4.60 ± 0.89 iu/L and 8.60 ± 0.89 iu/L respectively compared to 17.00 ± 0.71 iu/L of control. Day 14 also showed a significant ($P<0.05$) decrease of 12.60 ± 0.89 iu/L, 17.60 ± 0.89 iu/L and 16.40 ± 1.14 iu/L with doses 200 mg/kg, 400 mg/kg and 600 mg/kg respectively compared to 18.60 ± 0.89 iu/L of control. At day 21 with 200 mg/kg and 600 mg/kg extract doses, there was a significant ($P<0.05$) decrease of 12.60 ± 0.89 iu/L and 12.20 ± 0.84 iu/L respectively as compared to 25.80 ± 0.84 iu/L of control. Day 7 of extract withdrawal showed a significant ($P<0.05$) increase in ALT level with doses 200 mg/kg and 400 mg/kg, but 600 mg/kg of extract dose showed a significant ($P<0.05$) decrease of 26.20 ± 1.30 compared to 29.40 ± 1.14 iu/L of control.

ALP level at days 7, 14 and 21 all indicated significant ($P<0.05$) decreases as compared to the control group. Extract withdrawal at day 7 also showed a significant ($P<0.05$) decrease of (249.00 ± 0.71) with dose 400 mg/kg compared to 392.40 ± 1.52 iu/L control but there was a significant ($P<0.05$) increase of 483.60 ± 2.51 iu/L with dose 600 mg/kg compared to control of 392.40 ± 1.52 iu/L.

Discussion

This study was conducted to evaluate the effect of methanol extract of young pods of *Moringa oleifera* on biochemical and histopathological responses in Wistar rats.

Hypercholesterolemia is a risk factor for coronary artery disease (Tilkian et al., 1979). From the result of this study, treatment with the methanol extract of the young pod of *Moringa oleifera* indicated a significant ($p<0.05$) decrease in total cholesterol level at day 7; this result is in contrast with the result of Ola-Davies et al., (2014) who reported a non significant difference in Wistar rats treated with ethanol extract of *Moringa oleifera*. However, he treated the rats at lower dosages (50, 100 and 150 mg/kg). Lowered serum cholesterol enhances the rate of atherosclerosis resolution, thereby reducing the risk of cardiovascular disease (Gotto, 1997). Glycosides are complex organic substances that are known to exert pronounced physiological action even though they may be poisonous to man and animal (Frantisek, 1991). Cardiac glycosides are still the drug of choice for the treatment of congestive heart failure (Frantisek, 1991). The presence of cardiac glycosides secondary metabolite in the young pods of *Moringa oleifera* (Yahi et al., 2014) may be responsible for the reduction of cholesterol. Although the result of this study showed a significant ($p>0.05$) increase in the level of cholesterol at day 14, it may mean that prolonged usage or higher dosage may not be beneficial to the heart. Ola-Davies et al. (2014) reported a non-significant

difference at lower dosage.

Albumin is the most abundant protein found in the plasma, cerebrospinal fluid (CSF), urine and most extracellular fluid. It is synthesized exclusively in the liver and accounts for up to 69% of total protein. Its main function is the maintenance of colloid oncotic pressure in the intravascular and extravascular spaces. It also functions as a carrier protein to transport a large number of compounds including calcium and administered drugs as well as transport amino acids synthesized in the liver to other tissues (Kahn, 2005). Accurate assessment of albumin helps to detect hypoproteinemic disease such as liver failure, protein-losing nephropathy and protein-losing enteropathy. A true increase in albumin is pathognomonic for dehydration and decrease in liver failure, renal loss, polyuria (Kahn, 2005). The result of this work showed a significant ($P<0.05$) increase at day 14 with all doses of the extract while days 7 and 21 showed a significant ($P<0.05$) decrease of albumin level. This work is in agreement with the work of Tijani et al., (2016) who reported a significant decrease in the albumin level of broiler chickens fed different percentage of *Moringa oleifera* in their diets. Nonetheless our result differs with the report of Ola-Davies et al. (2014) who reported a non-significant difference in Wistar rats treated with ethanol extract of *Moringa oleifera*. Decrease in albumin may be primarily due to reduction in synthesis by the liver and secondarily to reduced protein intake which may indicate hepatic problem (Luskova et al., 2002; Jyotsna et al., 2003). This may suggest that the plant may probably exhibit a hepatoprotective or nephroprotective effects, at a moderate dose compared to a lower dose that may not have any effect or higher dosage may have a negative effect on the liver. This could be seen by the report of Ola-Davies et al. (2014) who showed a non-significant ($p>0.05$) difference in the level of albumin at lower dosages. Albumin apart from being a useful indicator of the integrity of glomerular membrane is also important in determining the severity of disease (Adedapo et al., 2005).

Hyponatremia was earlier associated with the hypotensive properties of the plant (Faizi et al., 1998). Sodium is the major cation of the extracellular fluid. It plays an important part in regulating acid-base balance, protecting the body against excessive fluid loss and preserving the normal function of muscle tissue (Tilkian et al., 1979). Day 7 of extract administration in this study indicated a decrease in serum sodium level which may suggest that the plant can be used in controlling hypertension due to hypernatremia. However, days 7 and 14 showed an increase in the sodium level, this may suggest that prolonged administration may

not be beneficial; on stoppage of treatment (withdrawal period) the level of sodium was seen to significantly ($p < 0.05$) decrease at 600mg/kg.

There was a decrease in serum potassium ion at day 7 of extract administration. Therefore the oral administration of the plant appears to be safe on cardiac tissue, since high level of K^+ in the blood may cause cardiac toxicity, including weakness of contraction and arrhythmia which may terminate in cardiac death (Groski and Gannon, 1979). Serum potassium levels are profoundly affected by momentary acid-base changes. Most often, significant hypokalemia reflects total body depletion of K^+ which has profound metabolic consequences (Tilkian et al., 1979). Hypokalemia was evident during the first 7 days of administration with all doses.

There was increase calcium level at day 14 of extract treatment. Calcium ion, apart from its importance in the maintenance of the skeletal structure of the body is involved in blood coagulation, functioning of the heart, muscles and nerves and permeability of cell membrane (Roberts et al., 2000). Renal failure can cause intravascular accumulation of metabolic waste such as urea and alteration in serum ion concentrations particularly sodium, calcium, potassium and phosphate (Abdulrahman, 2004).

Urea and creatinine are excellent indicators of protein metabolism and kidney function. In this study, there was a decrease in the level of urea and creatinine at day 7 of extract administration, while day 14 of extract administration indicated a significant ($P < 0.05$) increase with the same doses of extract, whereas, day 21 of treatment indicated a significant decrease. This may imply that there was a transient renal impairment probably due to prolonged or higher dosage administration followed by adaptation by the renal tissue. Tijani et al. (2016) also reported a significantly ($P < 0.05$) higher creatinine content in birds fed *Moringa oleifera* leaf meal -based diet at 20%; while Ola-Davies et al. (2014) reported a non-significant difference with lower dosages (50, 100 and 150 mg/kg) in rats treated with ethanol extract of *Moringa oleifera*. Creatinine is a waste product of creatine which is present in skeletal muscle as creatine phosphate, a high energy compound. Serum creatinine determination is one of the means of testing for renal function, reflecting the balance between its production (proportional to the body's muscle mass) and filtration by the renal glomerulus (Tilkian et al., 1979). From the biochemical results obtained in this study, there was no serious hepatic effect on the leakage enzyme following the extract administration even at dose 600 mg/kg. There was a decrease in serum aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) levels. This result agrees with the work of Tijani et al., 2016 who reported a significant ($p < 0.05$) decrease in the level of serum

enzymes (AST, ALT) in broilers fed varying percentage 5% to 20% of *Moringa oleifera* in their diets. At lower dosages Ola-Davies et al. (2014) reported a non-significant difference of liver enzymes (AST, ALT and ALP) in Wistar rats treated with ethanol extract of *Moringa oleifera*. The decrease in serum levels of these enzymes maybe due to hepatoprotective effect of the extract (Sodipo et al., 2013). Decreased liver enzymes have been reported with extract of *Veronia amygdalina* (Atangwho et al., 2007), *Cattaranthus roseus* (Iweala and Okeke, 2005) and *Solanum macrocarpum* (Sodipo et al., 2013).

The decrease in AST level at day 7 and 14 could also be that the extract is not injurious to the heart and the skeletal muscle. ALT is an enzyme found mainly in liver cells. A significant ($P < 0.05$) decrease in ALT level observed during days 7, 14 and 21 of extract administration with all doses administered, corroborated that of Pari and Kumar (2002) who reported that extracts of *M. oleifera* enhanced recovery from hepatic damage induced by antitubercular drugs. Mekonnen et al. (2005) in assessing cytotoxicity of *Moringa* extract in liver cells concluded that the aqueous extract of the leaves did not alter glutathione or lactate dehydrogenase levels or affect cell viability thus suggesting that it may not be toxic making it consistent with its use as a vegetable. ALP is an enzyme that mediates some of the complex reactions of bone formation. When osteoblasts are actively depositing bone matrix, they secrete large quantities of ALP. Since the two main sources of ALP are bone and liver, an elevation of alkaline phosphatase immediately directs attention to either liver problems or bone disease (Tilkian et al., 1979).

Since elevated levels of creatinine are found in renal dysfunction or muscle damage and urea is a waste product of protein breakdown (Mukherjee, 1988), it therefore implied that the extract had no deleterious effect on the kidney. Shi et al. (2004) has shown that high saponin diet has inverse relationship with renal stones. Since the phytochemistry revealed the presence of saponins in the methanolic extract of young pods of *Moringa oleifera* (Ojo et al., 2014) this may mean that the young pod may probably not cause renal damage.

Conclusion

It may be concluded that prolonged administration of methanolic extract of young pods of *Moringa oleifera*, increased serum albumin, decrease serum cholesterol, decrease serum sodium, increase potassium and calcium, decrease urea and creatinine levels and caused decrease in AST, ALT and ALP enzymes.

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Conflict of Interest

Authors wish to state that there are no known conflicts of interest associated with this work.

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