Saudi Journal of Medicine (SJM)
Scholars Middle East Publishers
Dubai, United Arab Emirates
Website: http://saudijournals.com/

ISSN 2518-3389 (Print)
ISSN 2518-3397 (Online)

The Lipidemic and Antioxidant Role of *Moringa oleifera* Leave Extract Following Salt Loading

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Article History
Received: 08.12.2017
Accepted: 16.12.2017
Published: 30.01.2018

DOI:
10.36348/sjm.2018.v03i01.002

Abstract: Salt consumption is essential but has a negative health impact when taken in excess. Therefore, this research is aimed at investigating the possible role of Moringa oleifera leave extract on lipids and antioxidant enzymes following salt loading. 24 male albino Wistar rats weighing between 180-240g were used for this study and were divided into four groups (n=6). They were given either normal rat feed and drinking water, high salt diet (8% NaCl diet) + 1% NaCl drinking water and/or *Moringa oleifera* extract (600 mg/kg b.w., orally, once daily). After six weeks of feeding, the animals were sacrificed and blood collected through standard method for analysis. The salt fed untreated rats had significant ($p<0.05$) increase in Triglycerides (TG) level, Atherogenic indices ($P<0.05$), Malondialdehyde (MDA), Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) ($P<0.01$). These parameters were significantly ($p<0.05$) reduced to near control values following extract treatment. Salt fed untreated rats were also observed to have significant ($P<0.05$) reduction in High density lipoprotein (HDL), Catalase (CAT) ($P<0.01$), Glutathione peroxidase (GPx) ($P<0.05$), Superoxide dismutase (SOD) and Albumin (ALB) ($P<0.01$), but the reverse was the case following extract treatment. In conclusion, consumption of *Moringa oleifera* leaf extract ameliorates the adverse effects of salt loading on the atherogenic, antioxidants and other indices thereby preventing possible outcome of cardiovascular disease, atherosclerosis and other related ailment. It is therefore being recommended for further research and possible use in the manufacture of drugs that are necessary in management of cardiac and other related ailments.

Keywords: *Moringa oleifera* Lam., Lipid profile, Atherogenic indices, antioxidants, Liver Enzymes.

INTRODUCTION

Moringa oleifera is a tree that is known to have originated from India, it is also being found in Sri-Lanka, Thailand, Pakistan, Phillipine, Indonesia, Taiwan, Haiti, South America, Caribbean and Africa (Nigeria) [10]. It is commonly known as Drumstic, [11] horseradish tree, ben oil (benzoil) [12], mothers best friend and Nebeday depending on location and there are about 13 species of Moringa belonging to Moringaceae family [13, 14].Almost all the plant parts are essential in the synthesis of therapeutic drugs [15], they also have important agricultural, commercial and economical values. Proximate and phytochemical analysis of *Moringa oleifera* leave extract [16] reveals its rich nutritive and bioactive components which include carbohydrate, fat, protein and minerals eg.calcium, iron, magnesium, manganese, phosphorus, potassium, sodium, zinc and water [12, 17, 18]. Others include vitamins (Vit. B1, B2, B3, B5, B6, B9, Vit. C. Vit. E and K), Carotenoids and antioxidants which include flavonoids, glycosides, terpinoids, zeatin [16], quercetin and kaempferol [12, 18]. Antioxidant components of *Moringa oleifera* like Vit. C, carotene and quercetins are known to play major roles in lowering blood pressure [19, 20, 21], quercetins and flavonoids can inhibit the production of nitric oxide and tumor necrosis factor by Kupffer cell when stimulated by injury [22] flavonoids also protect the cell against injury caused by x-ray and block the progression of the cell cycle and prostaglandin synthesis thereby inhibiting mutation and preventing carcinogenesis in experimental animals [23], the Vit. E antioxidant which is composed of tocopherol and alpha tocopherol is the most abundant and active component of this plant.This vitamins prevent lipid peroxidation chain reaction generated by free radicals from cellular and subcellular membrane which are rich in polyunsaturated lipid thereby preventing atherosclerosis and cancer [24, 25]. The Vitamin C components of *Moringa oleifera* can act as a scavenger of free radicals and do also regenerate Vitamin E indirectly [24], by virtue of this synergy, both vitamins C and E have attracted interest as agent that can retard atherosclerosis by reducing low density lipoproteins oxidation and thus preventing injury to the vascular endothelial cells [26],the antioxidant also protect against structural defect thus inhibiting free radicals formation [39].

Vitamin A is important for normal vision in dim light and for resistance against infection [24, 27, 28] the chlorogenic acid [29], and isothiocyanate component of Moringa oleifera also plays a major role in reducing blood sugar level in addition to its anti-inflammatory [26, 30, 31] anticancer and antimicrobial effect [32-34]. Moringa oleifera extract are also known to possess antitumor and hepatoprotective activities [30, 35], antispasmodic [14, 36, 37] and antiepileptic activities [38]. In addition, blood parameters like Red blood cell (RBC) count, packed cell volume (PCV), hemoglobin (Hb), white blood cell (WBC) counts, and platelets (Plt) were shown to be enhanced following consumption of *M. oleifera* [40] and even in situations of excessive salt consumption [41]. This research is therefore aim at investigating the possible role of Moringa oleifera leave extract on lipids and antioxidant enzyme following salt loading. This research was preconceive taking full cognizance of the fact that excessive dietary salt consumption over an extended period of time in our day to day life may predispose an individual to hypertension, cardiovascular disease, left ventricular hypertrophyand other opportunistic diseases, [1-7] it has also been shown to increase the risk of myocardial infarction, stroke, arterial stiffness and heart failure [8-9].

MATERIALS AND METHODS

Experimental-Animals

Twenty four (24) male albino Wistar rats weighing initially between 180 to250g obtained from the animal house of the Department of Physiology, University of Calabar, Nigeria were employed for this study for 6 weeks. The animals were allowed free access to their feed and drinking water. The rats were weighed before commencement of the feeding experiment and thereafter were weighed daily. Ethical approval was obtained from the Ethics Committee of the Faculty of Basic Medical Sciences, University of Calabar, Nigeria. They were nursed under control and the environmental conditions were in accordance with international standard [42].

Moringa oleifera extract preparation

The aqueous extract was prepared according to standard procedure [43]. Fresh leaves of *Moringa oleifera* were obtained from Calabar municipality, Cross River State and were identified by the Herbarium in Botany Department. The leaves were washed to remove debris and were later dried in an airy-room away from direct sunlight to avoid possible damage to their phyto-constituents for two days. The leaves were further oven- dried for 30 minutes at the temperature of 40^0C. The dried leaves were grinded to powdered form.

About 1400gram of the powdered *Moringa oleifera* leaf was soaked in 7000ml of distilled water for about 24 hours. The mixture was then filtered with a white cotton (satin) material, followed with filter paper (Whatmann No.1) into beakers and placed in an oven. The filtrate was evaporated to dryness using a rotary evaporator with temperature set at 50^0C. *Moringa oleifera* extract was then collected into a sample bottle and preserved in a refrigerator.

Salt diet and Drinking water

Salt feed containing 8% NaCl was prepared by mixing together 8g NaCl in 92g of the rat feed. Also 1% NaCl drinking water was prepared by dissolving 100g of NaCl in small quantity of distilled water and volume made upto10L with distilled water [45].

Experimental design

Twenty four (24) male albino Wistar rats weighing between 180 – 240g was randomly assigned into four (4) groups of six (6) rats each.

Group 1 (control) - received normal rat feed + drinking water

Group 2- received same as group 1 + *Moringa oleifera* extract (600mg/kg o.p. once daily)

Group 3- received 8% NaCl diet + 1% NaCl water

Group 4-received same as group 3 +*Moringa oleifera* extract (600mg/kg o.p. once daily).

The administration was done orally and the experiment lasted for a period of six weeks.

Collection of blood samples and analysis of different parameters

Blood samples were collected via cardiac puncture into EDTA capped bottles and the different parameters were analyzed thus.

Measurement of Lipid Profile

Measurement of total cholesterol

Total cholesterol was measured according to standard procedure [44].

Principle

Cholesterol esters are hydrolysed by cholesterol esterase to produce cholesterol and fatty acids. The cholesterol is oxidized by cholesterol oxidase to cholesterone and hydrogen peroxide. The H_2O_2 is later hydrolysed by peroxidase to form water and oxygen. The oxygen then reacts with 4-aminoantipyrine which is the chromogen to form quinoneimine. The colour intensity of the solution is proportional to the concentration of cholesterol in the sample.

The samples were mixed and incubated for 10 minutes in a water bath at 37^oC. The colour produced was read colorimetrically at 540nm.

Calculation

$$\frac{\text{Absorbance of test X concentration of standard}}{\text{Absorbance of standard}}$$

Measurement of triglyceride

Triglyceride was measured according to standard procedure [44].

Principle

Triglyceride in the sample was hydrolysed by lipoprotein lipase to glycerol and frees fatty acids. Glycerol is phosphorylated by the kinase to form glycerol-3-phosphate and ATP. The glycerol phosphate is then oxidized by glycerolphosphate oxidase to dihydroxyacetone phosphate and H_2O_2. The H_2O_2 is hydrolysed by peroxidase to form $H_2O$and O_2. The O_2 then react with 4-amino-antipyrine and phenol to form the colour complex quinoneimine.

The samples were mixed and incubated for 10 minutes in a water bath at 37^{o}C. The colour produced was read colorimetrically at 540nm.

Calculation

$$\frac{\text{Absorbance of test X Concentration of standard}}{\text{Absorbance of standard}}$$

Measurement of high density lipoprotein cholesterol

The High Density Lipoprotein was measured according to standard procedure [44].

Principle

HDL-cholesterol is a precipitate of apo protein B-containing lipoprotein using a mixture of sodium phosphotungstic acid and magnesium chloride.

The samples were mixed thoroughly and allowed to stand at room temperature for 15 minutes and later centrifuged at 3000 revolutions per minute.

The samples were mixed and incubated for 10 minutes in a water bath at 37^{o}C.

Calculations

$$\frac{\text{Absorbance of test X concentration of standard}}{\text{Absorbance of standard}}$$

Final result is multiplied by the dilution factor 3.0

Measurement of low and very low density lipoprotein

Low density and Very low density lipoprotein concentrations were measured based on Friedwald formular [46].

$$VLDL_C = \frac{\text{Triglyceride}}{2.22}$$

$$LDL_C = \text{Total Cholesterol} - (HDL_C + VLDL_C)$$

Measurement of Cardiac risk ratio (Atherogenic indices):

The different cardiac risk ratios were measured according to standard procedure.

Cardiac Risk Ratio (CRR) = Tc/HDL-c [47]

Atherogenic coefficient (AC) = (Tc – HDL-c) / HDL-c [48]

Atherogenic index of plasma (AIP) = log (TG / HDL-c) [49]

Measurement of Antioxidants

The different antioxidants were analyzed according to standard procedure. Glutathione Peroxidase (GPx) activity was determined using the Rice Evans method [50] Malondihaldihyde (MDA) was determined according to standard procedure [51]. Catalase (CAT) activity in the serum was determined as described by Abebi [52]. The change in the absorbance was monitored spectrophotometrically at 240nm over a 5min period.

Superoxide Dismutase (SOD) activity in the serum was determined by assessing the inhibition of pyrogallol-auto oxidation [53]. Changes in the absorbance at 40nm were recorded at 1min interval for 5min. SOD activity was determined from standard curve of % inhibition of pyrogallol auto-oxidation with SOD activity

Measurement of liver enzymes

Measurement of Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT)

AST and ALT were measured as described by Reitman and Frankel [54] and as usedby Archibong *et al*. [55].

Principle

AST hydrolysed aspartic acid and α-ketoglutarate substrate to give oxaloacetic acid and glutamate. The oxaloacetic acid is spontaneously decarboxylated to form pyruvate which reacts with 2,4-dinitrophenylhydrazine(DPNH) to give a brown-coloured hydrozone, which is read in the colorimeter at 540nm [54].

ALT reacts with alanic acid and α-ketoglutarate to yield pyruvate and glutamate. The pyruvate formed reacts with 2, 4-dinitrophenylhydrazine (DPNH) to give a brown-coloured hydrazone, which is read in the colorimeter, at 540nm [54].

Each test tube containing the sample was incubated in a water bath for 30 minutes. The test-tubes were then removed and kept on a working bench. 0.5ml of 2.4 – nitrophenylhydrazine was added to each test tube, mixed thoroughly and allowed on the bench for 20 minutes. 0.4N NaOH was added to stop the reaction which was read colometrically at 540nm.

Calculations

$$\frac{\text{Absorbance of test} \times \text{Concentration of AST standard}}{\text{Absorbance of standard}}$$

Measurement of alkaline phosphatase (ALP)

ALP was measured using standard method [83].

Principle

P- nitrophenyl phosphate is hydrolysed by alkaline phosphate to give phosphate and p-nitrophenol. The p-nitrophenol complex formed is read colometrically at 405nm.

The samples were mixed thoroughly and absorbance read immediately.

Calculations

$$\frac{\text{Absorbance of test} \times \text{Concentration of standard}}{\text{Absorbance of standard}}$$

Measurement of Serum Proteins

Total protein

The total protein was measured using the Burette method as described by Tiez [56]

Principle

The peptide bond in protein has affinity for burette reagent. In alkaline medium, $CuSO_4$ in the burette reagent reacts with the peptide bond of protein to give a blue colour complex. The colour produce is proportional to the concentration of protein in the sample. The samples were thoroughly mixed and incubated in a water bath at 37^oC for 10 minutes. After which they were then removed from the water bath and read colometrically at 540nm [56].

Serum albumin

The serum albumin was measured using the Bromocresol green method as described by Grant [57]

Principle

In an acidic medium bromocresol green dye binds to albumin to give a violet green colouration. Samples were mixed thoroughly and incubated at room temperature for 5 minutes and read colometrically at 620nm [57].

Globulin

Calculation: Total Protein – Albumin = Globulin

STATISTICAL ANALYSIS

Data are presented as mean ± SEM. Data were analyzed using one-way analysis of variance (ANOVA) and then followed by post hoc test (least square deviation). Data analysis was done with the help of computer software (Excel and SPSS version 17.0 for windows). P-values of less than 0.05 were considered as significant.

RESULTS AND DISCUSSIONS

Lipid profile

As shown in Table 1 the difference in total cholesterol concentration in the *M. oleifera* group (181.58±4.88), Salt fed group (206.04±12.21) and Salt fed + *M. oleifera* group (222.58±24.04) was of no statistical significance when compared with the control group (208.11±8.81) respectively. Triglyceride concentration in the salt fed groups (27.61±0.46) was significantly increased ($P< 0.05$) when compared with the control (23.25±1.74) and M. oleifera (24.95±1.13,) groups respectively but treatment with the extract reduced the TG concentration in salt fed group as indicated by its concentration in the salt fed + *M. oleifera* group (25.73±1.02).

High density lipoprotein (HDL) concentration in the salt fed group (17.53±1.09) was significantly decreased ($p<0.05$ and $p<0.001$) when compared with that of control (21.85±1.01) and *M. oleifera* (24.53±1.95) groups respectively but treatment with the extract increased the concentration of HDL concentration in the salt fed group as indicated by its concentration in the salt fed + *M. oleifera* group (21.05±0.54).

The difference in Low density lipoprotein (LDL-c) concentration in the *M. oleifera* groups (151.14±4.69), salt group (182.99±12.12) and Salt fed + *M. oleifera* (196.39±24.25) was of no statistical significance when compared with the control group (181.68±9.20).

The very low density lipoprotein concentration(VLDL-c) in the *M. oleifera* group (5.91±0.61) was significantly higher ($P<0.05$) than that of the control group (4.58±0.37), salt fed group (5.52±0.09) and Salt fed + *M. oleifera* group (5.15±0.20) respectively

Cardiac risk indices

As shown in Table 2, the cardiac risk ratio in the salt fed group (11.93±0.96) was significantly increased ($p<0.05$ and $p<0.01$) when compared with the control (9.71±0.84) and *M. oleifera*(7.62±0.58)groups respectively but treatment with the extract decreased the CRR in the salt fed group as indicated by its value in the salt fed + *M. oleifera* group (10.63±1.21)

The atherogenic coefficient in the salt fed group(10.93±0.96) was significantly increased ($p<0.05$ and $p<0.01$) when compared with the control(8.71±0.84) and *M. oleifera* (6.62±0.58) groups respectively but treatment with the extract decreased the atherogenic coefficient in the salt fed group as indicated by its value in the salt fed + *M. oleifera* group (9.63±1.21)

The atherogenic index in the salt fed group (0.20±0.02) was significantly increased ($p<0.05$ and $p<0.001$) when compared with the control (0.02±0.05) and *M. oleifera* (0.01±0.04) groups respectively but treatment with the extract decreased the atherogenic index in the salt fed group as indicated by its value in the salt fed + *M. oleifera* group (0.09±0.02).

Antioxidant Enzymes

As shown in Table 3, catalase (CAT) level in the salt fed group (0.27±0.03) was significantly decreased ($p<0.01$ and $p<0.001$) when compared with control (0.37±0.01) and *M. oleifera* (0.41±0.02) groups respectively but treatment with the extract increased the Catalase level in the salt fed group as indicated by its value in the salt fed + *M. oleifera* group (0.30±0.03).

The Glutathione Peroxidase (GPx)level in the salt fed (850.67±42.33) group was significantly decreased ($p<0.01$) when compared with control (946.69±31.17) and *M. oleifera*(1057.67±53.36) groups respectively but treatment with the extract increased the GPx level in the salt fed group as indicated by its value in the salt fed + *M. oleifera* group (888.17±36.84)

The Superoxide dismutase (SOD)level in the salt fed (0.12±0.02) group was significantly decreased ($p<0.001$) when compared with control (0.22±0.02) and *M. oleifera* (0.23±0.02) groups respectively but treatment with the extract increased the SOD level in the salt fed group as indicated by its value in the salt fed + *M. oleifera* group (0.16±0.01).

The malondialdehyde (MDA)level in the salt fed (15.33±0.84) group was significantly increased ($p<0.01$) when compared with control (12.00±0.77) and *M. oleifera* (11.67±0.49) groups respectively but treatment with the extract decreased the MDA level in the salt fed group as indicated by its value in the salt fed + *M. oleifera* group (13.80±0.83).

Liver Enzymes

As shown in Table 4theAspartate Transferase (AST) level in the salt fed group (59.28±4.10) was significantly increased ($p<0.01$ and $p<0.001$) when compared with that of control (43.16±2.08) and *M. oleifera*(46.25±0.25) groups respectively but treatment with the extract decreased the AST level in the salt fed group as indicated by its value in the salt fed + *M. oleifera* group (47.69±2.31).

Alanine Transferase (ALT) levelin the salt fed group (70.33±3.91) was significantly increased ($p<0.001$) when compared with that of control (47.28±3.26) and *M. oleifera* (45.54±3.82) groups respectively but treatment with the extract decreased the ALT level in the salt fed group as indicated by its value in the salt fed + *M. oleifera* group (49.13±2.10)

Alkaline Phosphatase (ALP) levelin the salt fed group (44.76±3.17) was significantly decreased ($p<0.01$) when compared with that of control (47.41±2.51)but treatment with the extract further decreased the ALP level in the*M. oleifera* (35.69±3.06) and salt fed + *M. oleifera* group (42.51±0.83).

Serum Protein

As shown in Table 5, the total protein concentration in the salt fed group (60.17±0.80) was significantly decreased ($p<0.05$ and $p<0.01$) when compared with that of control (64.33±0.80) and *M. oleifera* (64.17±0.40) groups respectively but treatment with the extract decreased the total protein level in the salt fed group as indicated by its value in thesalt fed + *M. oleifera* group (62.00±0.73).

The Albumin levelin thesalt fed group (26.33±0.61) was significantly decreased ($p<0.001$) compared with that of*M. oleifera*(34.00±0.73) and control group (34.33±0.61) but treatment with the extract increasedthe albumin level in the salt fed group as indicated by its value in the salt fed + *M. oleifera* group (30.00±0.73).

Globulin level in the salt fed group (34.17±0.17) was significantly increased ($p<0.001$) when compared with that of control (30.00±0.42) and *M. oleifera* (30.17±0.49) groups respectively but treatment with the extract decreased the globulin level in the salt fed group as indicated by its value in the salt fed + *M. oleifera* group (32.50±0.62).

Table-1: Showing comparison of Lipid profile in the different groups

	Tc	TG	HDLc	VLDLc	LDL
Control	208.11±8.81	23.25±1.74	21.85±1.01	4.58±0.37	181.68±9.20
M. oleifera	181.58±4.88	24.95±1.13	24.53±1.95	5.91±0.61*	151.14±4.69
Salt Fed	206.04±12.21	27.61±0.46*	17.53±1.09*	5.52±0.09	182.99±12.12
Salt Fed + *M. oleifera*	222.58±24.04	25.73±1.02	21.05±0.54	5.15±0.20	196.39±24.25

Values are represented as Mean ± SEM. *$p<0.05$ vs Control.

Table-2: Showing comparison of Atherogenic indices in the different groups

	CRR	AC	AIP
Control	9.71±0.84	8.71±0.84	0.02±0.05
M. oleifera	7.62±0.58	6.62±0.58	0.01±0.04
Salt Fed	11.93±0.96	10.93±0.96	0.20±0.02*
Salt Fed + *M. oleifera*	10.63±1.21	9.63±1.21	0.09±0.02*

Values are represented as Mean ± SEM. *p<0.05 vs control.

Table-3: Showing comparison of Antioxidant enzymes in the different groups

	MDA	CAT	GPx	SOD
Control	12.00±0.77	0.37±0.01	946.69±31.17	0.22±0.02
M. oliefera	11.67±0.49	0.41±0.02	1057.67±53.36	0.23±0.02
Salt Fed	15.33±0.84**	0.27±0.03**	850.67±42.33	0.12±0.02***
M. oleifera + Salt fed	13.83±0.83	0.30±0.03*	888.17±36.84	0.16±0.01*

Values are represented as Mean ± SEM. *p<0.05, **p<0.01, ***p<0.001 vs control.

Table-4: Showing comparison of liver enzyme in the different groups

	AST	ALT	ALP
Control	43.16±2.08	47.28±3.26	47.41±2.51
M. oleifera	43.25±0.25	45.54±3.82	35.69±3.06
Salt Fed	59.28±4.10***	70.33±3.91***	44.76±3.17***
Salt fed + *M. oleifera*	47.69±2.31	49.13±2.10	42.51±0.83

Values are represented as Mean ± SEM. ***p<0.001 vs control

Table-5: Showing comparison of Plasma Protein in the different groups

	Total Protein	Albumin	Globulin
Control	64.33±0.80	34.33±0.61	30.00±0.42
M. oleifera	64.17±0.40	34.00±0.73	30.17±0.49*
Salt Fed	60.17±0.40**	26.00±0.61	34.17±0.17***
Salt Fed + M. Oleifera	62.00±0.73***	30.00±0.73***	32.50±0.62***

Values are represented as Mean ± SEM. *p<0.05, **p<0.01, ***p<0.001 vs control

The result as obtained from this investigation is quite amazing and shows to a large extent the potent effect of *M. oleifera* extract.

The lipid profile analysis revealed that extract of *M. oleifera* was able to significantly reverse the increase in TG and the decrease in HDL-c evidenced in salt loaded rats. This result is further corroborated by earlier researches by Mehta *et al.*, [84, 85] which shows that extract of M. oleifera was able to bring about increase in HDL but decrease in TG, vLDL, LDL and atherogenic indices. Raised levels of serum total cholesterol, triglycerides and low density lipoprotein cholesterol are possible indication of coronary heart attack, risk of heart disease and stroke [58]. This result is of significance because accumulation of LDL-c predisposes one to cardiovascular disease, but on the contrary accumulation of HDL-c prevents against cardiovascular disease. The ability of *M. oleifera* leaf extract to reduce these bad cholesterols in the blood maybe unconnected with the fact that leaf extract of *M. oleifera* contain omega-3 and omega-6 fatty acid [59] which is believed to mediate the decrease in the concentrations of bad cholesterol thereby preventing atherosclerosis and related cardiovascular diseases. The increase in HDL-c observed in the *M. oleifera* treated groups could also be attributed to omega 3 component of the extract [60, 61], which is equally important because HDL-c is the good cholesterol that function in preventing the accumulation of bad cholesterol and ameliorating the risk of heart disease. It has also been shown that low concentration of HDL increases the risk of atherosclerotic disease therefore individuals with seemingly low level of LDL also stand the same risk in situations of inadequate HDL [62 - 64].

It is also important to note that alpha tocopherol which is the most abundant active component of this plant functions effectively in preventing lipid peroxidation chain reaction generated by free radicals from cellular and subcellular membrane which are rich in polyunsaturated lipid thereby preventing atherosclerosis and cancer [24, 25]. The Vitamin C components of *Moringa oleifera* can act as a scavenger of free radicals and do also regenerate Vitamin E indirectly [24], by virtue of this synergy, both vitamins C and E have attracted interest as agent that can retard atherosclerosis by reducing low density lipoproteins oxidation and thus preventing injury to the vascular endothelial cells [26], Therefore the ability of *M. oleifera* leaf extract to reduce these bad cholesterols following salt loading

shows that their consumption would be beneficial to health.

Atherogenic index is a strong marker that predicts the risk of atherosclerosis and coronary heart disease [89], it reflects the true relationship between protective and atherogenic lipoprotein and is associated with the size of pre and anti atherogenic lipoprotein particles [90]. A decrease in atherogenic index as shown by the M. oleifera extract treated group shows its potent health effect. This is because a lower value for atherogenic indices is associated with lower risk of cardiovascular diseases but a higher value indicate higher risk [90, 91]This is further being supported by earlier research study by Emmanuel *et al.* [84, 86] which shows that extract of M oleifera reduceselevation in cardiac risk ratio. This point goes a long way to explain the reason why administration of the extract brought about an increase in HDL.

Following the lipid profile result we decided to further investigate the effect of this extract on antioxidant enzymes following salt loading. The antioxidant result revealed that there was a significant decrease in catalase and superoxide dismutase activities caused by salt loading. But this was significantly reversed following combine treatment with *M. oleifera* extract. Also the abnormal increase in Malondialdehyde caused by salt loading was significantly reversed following the administration of *M. Oleifera* extract. This result is consistent with earlier findings by Bonoy *et al.* [65] which shows that extract of *M. oleifera* decreased MDA concentration while bringing about increase in CAT, SOD and GPx concentrations when administered alongside with high salt in rats [87, 88]. MDA is an important Marker for oxidative stress and lipid peroxidation [66, 67], that its activities were significantly increased in the salt fed group reveals to what extent there was peroxidation also ROS and free radicals may have been generated showing how detrimental excessive salt intake could be to the system.

CAT, SOD and GPx are important antioxidant enzymes that aids in the scouting and degrading of free radicals. SOD perform important function by catalyzing the conversion of superoxide radicals to O_2 and H_2O_2 [68, 69] while Catalase further decomposes the toxic H_2O_2 to O_2 and H_2O [70]. GPx is also known to catalyze the reduction of hydroperoxide by glutathione [71], it also protects against oxidative stress. That the level of antioxidant enzymes was reduced following Salt loading maybe unconnected with the fact that most of the antioxidant enzymes must have been used up in the degrading of the free radicals that were hitherto generated as a result of excessive salt ingestion or their production may have been inhibited following salt loading. Consequently the antioxidant enzyme activities were shown to have appreciated immensely following the administration of *M. oleifera* extract, as evident by the results. This edible plant leaves have been shown to possess important antioxidant components [13, 72] eg flavonoids, terpinoids, [16], quercetin and kaempferol [12, 18] which are believed to have boosted the activities of the antioxidant enzymes [73] as experienced by the M. oleifera + Salt treated group. Quercetins and flavonoids can inhibit the production of nitric oxide and tumor necrosis factor by Kupffer cell when stimulated by injury [22] flavonoids also protect the cell against injury caused by x-ray and block the progression of the cell cycle and prostaglandin synthesis thereby inhibiting mutation and preventing carcinogenesis in experimental animals [23], The Vitamin C and E components of *Moringa oleifera* can act as a scavenger of free radicals [24].Therefore this result goes a long way to proof how usefull *M. oleifera* can be in maintaining good health.

The serum enzyme results revealed that the hepatocytes or liver tissues benefited positively from the *M. oleifera* extract administration. This is because the increase in ALT and AST caused by ingestion of excess salt as revealed by the result was significantly reduced following the administration of *M. oleifera* extract. This result is further corroborated by earlier researches published by Ezejindu *et al*. and Afzal *et al*. [77,92], which shows that M. oleifera has a protective effect against liver injury. It is important to note that AST, ALT and ALP are biomarkers for healthy liver [74] and they play major role in the diagnosis of heart and liver disease including infections, [75] therefore any abnormal increase in AST, ALT and ALP is suggestive of tissue damage [76]. That this extract of *M. oleifera* was able to reverse the increase in liver enzymes occasion by salt loading, point to the ability of the extract to prevent hepatic damage as reported by Ezejindu *et al.*[77]. Liver protective herbal drugs contain a variety of chemical constituents like phenols, coumarins, lignans, essential oil, monoterpenes, carotinoids, glycosides, flavonoids, organic acids, lipids, alkaloids and xanthenes [93], most of these are present in the Moringa oleifera [17] leaves extract and so may be responsible for this beneficial effect. We can infer that leave extract of Moringa oleifera has an appreciable ability to prevent damage to the liver, therefore its ingestion may be of immense benefit to health in general.

The serum protein result shows that *M. oleifera* leaf extract was able to significantly reverse the decrease in total protein occasion by excessive ingestion of salt. This result is in consistence with findings by Dharmendra *et al*., [78] which revealed that administration of *M. oleifera* was able to increase total protein level. This increase in total protein concentration may be due to enhanced protein synthesis in the hepatic cells arising from inhibition of lipid peroxidation [79]. The globulin level was decreased following extract administration. This is of significant because elevated globulin concentration in plasma is an indication of stimulation of the immune system [80,

81]. The serum albumin result reveals that no destruction on the hepatocytes [82] was observed following moringa extract administration but the reverse was the case following salt loading, this result is very essential since albumin levels has been shown to be lowered in chronic and acute liver disease by Burtis *et al*.,[81]. Therefore that decrease in albumin level following salt loading was reversed following moringa extract administration is an indication that this extract may possess certain components that may prevent the liver from being destroyed by excessive salt ingestion.

CONCLUSION

In conclusion consumption of Moringa oleifera leaf extract ameliorates the adverse effects of salt loading on the atherogenic, antioxidant enzymes and other indices thereby preventing possible outcome of cardiovascular disease, atherosclerosis and other related ailment. It is therefore being recommended for further research and possible use in the manufacture of drugs that are necessary in management of cardiac and other related ailments.

ACKNOWLEDGEMENTS

The authors of this article do sincerely appreciate the effort of all those who supported this research in different ways. We want to say a big thank you to Mr. Ededet Umoh of Physiology Department who helped to supply the rats, breed and made them available for sacrifice and also to Mrs. Irene Bassey who made all the reagents and equipment available for use during the course of the study. Finally we want to thank the head of department of Physiology for allowing us to use the laboratory and other facilities for the study

AUTHORS CONTRIBUTION

This work was carried out in collaboration between all authors. Author ANA wrote the first draft of the manuscript, managed the literature and performed the statistical analysis, author CON and AUI designed the study, wrote the protocol and edited the manuscript, while authors AAA and KSE contributed in carrying out the feeding regimens and analysis of blood samples. All authors read and approved the final manuscript.

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