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Moringa oleifera Enriched Diet Ammeliorates the Toxic Effects of Palm Wine and Local Gin (Ogogoro) on Some Biochemical Parameters in Albino Rats

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Authors' contributions

This study was carried out in collaboration between all authors. Author OO designed the study, performed the statistical analysis, designed the protocol and wrote the first draft of the manuscript. Author KTA supervised and managed the analyses of the study, wrote the final draft manuscript. Author OBO managed the literature searches and validated all the references. All authors read and approved the final manuscript.

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ABSTRACT

The effect of *Moringa oleifera* enriched diet on the toxic effects of palm wine and local gin on liver enzymes, lipid profile and electrolytes in albino rats was determined in this research.
Methodology: Thirty female albino wistar rats, aged 8 to10 weeks, weighing between 170 and 200 g were used for the study. They were divided into six groups of five rats each. Group 1 served as the control group (given normal diet), group 2 rats were given *Moringa oleifera* enriched diet only, *g*roup 3 rats were given 10 ml/kg bwt of palm wine only and fed a normal diet, group 4 rats were given 0.03 ml/kg bwt of local gin and fed a normal diet, group 5 rats were given 10 ml/kg bwt palm

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wine with *Moringa oleifera* enriched diet and group 6 rats were given 0.03 ml/kg bwt of local gin with *Moringa oleifera* enriched diet. Palm wine and local gin were administered to the rats daily using an oral cannula for 30 days. On the 31st day, the rats were sacrificed and blood was collected through cardiac puncture for the determination of some liver enzymes, lipid profile and electrolytes.

Results: The results of this study showed that *Moringa oleifera* enriched diet significantly decreased ($p<0.05$) the activities of alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase, and significantly increased ($p<0.05$) the levels of total protein and albumin when compared with the control rats. Also, *Moringa oleifera* enriched diet caused significant decrease ($p<0.05$) in the levels of total cholesterol, triglycerides, low density lipoprotein-cholesterol and very low density lipoprotein-cholesterol; with significant increase ($p<0.05$) in high density lipoprotein-cholesterol when compared to control rats. Treatment with the extract significantly lowered ($p<0.05$) the levels of urea and creatinine in Groups 3, 4 and 5 rats compared with Group 2 rats. Furthermore, the significant reduction ($p<0.05$) in the levels of sodium, potassium, chloride and bicarbonate was reversed by, treatment with *M. oleifera* enriched diet.

Conclusion: The results suggest that the *Moringa oleifera* enriched diet could possibly ameliorates the toxic effects of palm wine and local gin on some biochemical parameters in rats.

Keywords: Moringa oleifera enriched diet; palm wine; local gin; electrolytes; liver enzymes; lipid profile; rats.

1. INTRODUCTION

Moringa oleifera is the most widely distributed species of the *Moringaceae* family throughout the world, especially in Asian countries, having a remarkable range of pharmacological properties in addition to significant nutritional value and hence have been scientifically evaluated for its possible medicinal applications [1]. It is a highly valued plant, distributed in many countries of the tropics and subtropics. It has an impressive range of medicinal uses with high nutritional value. Traditionally, *Moringa oleifera* is considered to be one of the most useful trees in the world, as almost every part of the tree has some nutritional, medicinal and other valuable properties [2].

Various parts of the plant such as the leaves, roots, seeds, bark, fruits, flowers and immature pods act as cardiac and circulatory stimulants, possess anti tumour [3], anti pyretic, anti epileptic, anti inflammatory, antiulcer properties [4]. Other important medicinal properties of the plant include antispasmodic [5], diuretic [6], antihypertensive [7], cholesterol lowering [8], antioxidant, antidiabetic, hepatoprotective [9], antibacterial and antifungal activities [10]. *M. oleifera* parts are being employed for the treatment of different ailments in the indigenous system of medicine, particularly in South Asia [11]. It has also been shown that *Moringa oleifera* leaf diet improves the toxic effects of palm wine and local gin on haematological and haemostatic parameters in albino rats [12]. In addition, *M. oleifera* seeds possess water purifying powers [13,14] by flocculating Gram– positive and Gram – negative bacteria cells [14,15,16]. It can also be used as a less expensive bio absorbent for the removal of heavy metals [17]. The leaves have also been reported to be a good sources of carbohydrate, lipids, proteins, minerals, vitamins carotenoids, flavonoids, terpenes and phytosterols [18].

Palm wine (alcohol) consumption is a major factor in the global burden of disease and should be considered a public health priority globally, regionally, and nationally for the vast majority of countries in the world [19,20,21]. Alcohol consumption has been linked to more than 60 medical conditions, and is also linked to categories of disease whose relative impact on the global burden is predicted to increase [22]. It has been shown to be associated with deleterious effects on reproductive functions, prenatal osteo-inhibitory effects, blood plasma protein malnutrition, multivitamin deficiencies and pre mature death [23,24,25].

Local gin (Ogogoro) is an alcoholic beverage prepared by the distillation of the fermented sap of Raphia palms (*Raphia hookeri*), coconut palm (*Cocus-nucifera*), and oil palm (*Elaeis guinesis*). It is popular in West Africa. It has widespread direct and indirect effects on the haematological system. Leukocyte, erythrocyte and thrombocyte production and functions are affected directly. Liver damage which is secondary to alcohol abuse adversely impacts red blood cells and the

haemostatic mechanisms [26]. Nutritional deficiencies are also caused by the effect of alcohol on the absorption, storage and utilization of several vitamins [27].

The potential effect of *Moringa oleifera* diet on palm wine and local gin deleterious effect on liver function, lipid profile and electrolytes was researched in the present study.

2. MATERIALS AND METHODS

2.1 Collection and Preparation of Leaves

Fresh mature healthy and of good quality *Moringa oleifera* leaves were collected from a farm in Okuku, Yala Local Government Area of Cross River State, Nigeria during the month of June, 2014. The plant material was botanically identified and authenticated (Herbarium number: RUBL-20287) in the Department of Botany, Faculty of Basic Medical Sciences University of Calabar, Nigeria. The leaves were thoroughly washed, shade dried for 14 days. The leaves were turned over to prevent mould growth and heat buildup that may change leaf colour. The completely dried and crispy leaves were ground in a domestic corn mill into powder and stored in air tight container until they were ready to be used in the *Moringa oleifera* enriched diet production.

2.2 Preparation of *Moringa oleifera* Enriched Diet

Standard rat pelleted diet was used as control diet. The pellet was ground to facilitate the incorporation of *Moringa oleifera* leaf powder. *Moringa oleifera* supplemented feed was prepared by mixing 11.1% on weight basis of *Moringa oleifera* leaf powder to normal feed. Dried *Moringa oleifera leaf* powder was weighed and mixed with rat chow in the ratio (1: 9, w/w respectively).

Mass of *Moringa oleifera* leaf powder = (x/100) x 35 kg = Y kg

Total feed weight per group = 35Kg.

Where

x = percentage of *Moringa oleifera* leaf powder needed in the diet.
Y = mass of *Moringa oleifera* leaf powder added.

These quantities were weighed and thoroughly mixed in an electrical mixer to produce the needed percentage of *Moringa oleifera* enriched diet. No heat treatment was applied.

2.3 Palm Wine and Local Gin (Ogogoro)

Fresh 50% diluted palm wine obtained from oil palm was collected on daily basis from a local palm wine seller, in Okuku Yala Local Government Area, Cross River State, Nigeria. The palm wine was administered at a dose of 10 ml/kg-bwt [22]. The local gin was administered at a dose of 0.03 ml/kg bwt [28].

2.4 Experimental Design

Thirty female albino rats were used for this study. The rats were aged 8 to10 weeks and weighed between 170 g and 200 g. They were divided into six groups: Groups1 to 6 of 5 rats each. Rats in each group were placed in separate cages in the Animal House of Cross River State University of Technology, Nigeria under natural day and night cycles. The rats had free access to normal feed and tap water *ad libitum*. They were allowed two weeks of acclimatization to their environment. The animals were subsequently treated as follows;

Group 1: Control; rats in this group were given 2 ml/kg bwt of distilled water and fed a normal diet.

Group 2: Rats in this group were fed *Moringa oleifera* enriched diet only.

Group 3: Rats in this group were given 10 ml/kg body weight of palm wine only and fed a normal diet.

Group 4: Rats in this group were given 0.03 ml/kg body weight of local gin only and fed a normal diet.

Group 5: Rats in this group were given palm wine (10 ml/kg) bwt and fed *Moringa oleifera* enriched diet.

Group 6: Rats in this group were given local gin (0.03 ml/kg) and fed *Moringa oleifera* enriched diet.

The palm wine and local gin were administered to the rats daily using an oral cannula. Administrations were done for a period of 30 days, while the rats were maintained on the appropriate diets.

2.5 Determination of Serum Liver Enzymes, Lipid Profile and Electrolytes

After a 24 hour fast, the rats were sacrificed and blood carefully obtained via direct cardiac puncture. The collected blood was transferred into plain tubes and centrifuged. Serum was appropriately obtained for the determination of the various parameters: alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total cholesterol, triglycerides (TG), low density lipoprotein cholesterol (LDL-C), and high density lipoprotein cholesterol (HDL-C) concentration using commercial kits obtained from Randox Laboratories, UK.

Very low density lipoprotein was calculated using this formula;

$$\text{VLDL (mg/dl)} = \frac{\text{Serum triglycerides}}{5}$$

2.6 Statistical Analysis

Data were presented as Mean ± SEM. Statistical significance was determined using the one way analysis of variance. Differences were considered statistically significant at a p value less than 0.05.

3. RESULTS

3.1 Effects of *M. oleifera* Enriched Diet on Liver Enzymes in Rats Treated with Palm Wine and Local Gin

The effects of *Moringa oleifera* enriched diet on the assayed liver enzymes: alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) is as shown in Table 1. Administration of the *Moringa oleifera* diet caused a significant reduction in the levels of alkaline phosphatase (ALP) in Groups 2, 5 and 6 compared to in group 1 (Table 1).

It also caused a significant reduction in the levels of alanine aminotransferase (ALT) in groups 2 and 6 compared to control group (Group 1).

A significant reduction ($p<0.05$) in levels of aspartate aminotransferase (AST) was observed in groups 2 and 6 rats as compared to group 1 rats. A significant increase ($p<0.05$) in the levels of total protein and albumin was observed in groups 2,5 and 6 rats as compared to group 1 rats (Table 1).

3.2 Effect of *M. oleifera* Enriched Diet on Lipid Profile in Rats Treated with Palm Wine and Local Gin

Table 2 shows the effect of *M. oleifera* enriched diet on lipid profile in rats treated with palm wine and local gin. The *Moringa oleifera* enriched diet caused a significant reduction ($p<0.05$) in the values of total cholesterol, triglyceride, low density lipoprotein cholesterol and very low density lipoprotein in Groups: 2 and 6 as compared to Group 1 rats); the diet caused a significant elevation ($p<0.05$) in the values of high density lipoprotein cholesterol in Groups 5 and 6 as compared to group 1 rats.

3.3 Effect of *M. oleifera* Enriched Diet on Electrolytes in Rats Treated with Palm Wine and Local Gin

The effect of *M. oleifera* diet on electrolytes in rats treated with palm wine and local gin is as shown in Table 3.

Palm wine and local gin caused a significant decrease ($p<0.05$) in sodium, potassium, chloride and bicarbonate in groups 3 and 4 rats when compared with group 1 rats. *Moringa oleifera* diet caused a significant increase ($p<0.05$) in the values of sodium, potassium and bicarbonate in group 2 compared with group 1 rats.

4. DISCUSSION

The present study was carried out to determine the effect of *Moringa oleifera* diet on some biochemical parameters in albino wistar rats exposed to palm wine and local gin. A significant increase in the activities of ALP, ALT and AST were elicited in the study; this is in accordance with [29]. Most experiments involving the induction of liver injury is usually accompanied by the elevation in the activities of liver enzyme markers (AST, ALT and ALP).

ALT is a cytoplasmic enzyme found in very high concentration in the liver and an increase of this specific enzyme indicates hepatocellular damage, while AST is less specific than ALT as an indicator of liver function [30]. An increase in the enzyme activity indicates cellular leakage and loss of functional integrity of the hepatic cell membrane [31,32]. The elevated levels of these biochemical parameters are direct reflection of alterations in the hepatic structural integrity [33].

Table 1. Effect of *Moringa oleifera* enriched diet on liver enzymes activities in rats treated with palm wine and local gin

Groups	Alkaline phosphatase (ALP) (IU/L)	Alanine aminotransferase (ALT) (IU/L)	Aspartate transaminase (AST) (IU/L)	Total protein (gm/dL)	Albumin (gm/dL)
Group 1: Positive Control.	44.77±3.46	27.32±1.97	83.35±0.84	7.31 ± 0.00	3.51 ± 0.00
Group 2: *Moringa oleifera* diet only.	29.98±2.90*	22.68±3.04*	75.15±3.03	7.69 ± 0.00*	5.34 ± 0.02*
Group 3: Palm wine only.	53.90±4.16	27.54±1.77	87.39±0.08	7.20 ± 0.00*	3.20 ± 0.03*
Group 4: Local gin only.	58.02±0.48	27.48±3.18	87.80±0.38	7.31 ± 0.00	3.02 ± 0.01*
Group 5: Palm wine +*Moringa oleifera* diet.	33.25±5.64*	38.30±9.68	82.38±0.30	7.29 ± 0.01*	4.05 ± 0.01*
Group 6: Local gin +*Moringa oleifera* diet.	35.81±5.28*	25.33±2.89*	76.87±3.23*	7.24 ± 0.01*	4.52 ± 0.01*

*All values=Mean±SEM; * significantly different from values of Group 1 at p<0.05*

Table 2. Effects of *Moringa oleifera* enriched diet on lipid profile in rats treated with palm wine and local gin

Groups	Total cholesterol (mmol/L)	Triglyceride (mmol/L)	High density lipoprotein (mmol/L)	Low density lipoprotein (mmol/L)	Very low density lipoprotein (mmol/L)
Group 1: Positive Control.	134.73 ± 2.07	146.70 ± 4.30	24.00 ± 1.67	63.78±0.46	26.95±0.86
Group 2: *Moringa oleifera* diet only.	124.56 ± 2.45	122.16 ± 8.56*	48.10 ± 2.25	52.03 ± 1.51*	24.43±1.71*
Group 3: Palm wine only.	145.17 ± 1.54	158.23 ± 0.78*	10.21 ± 0.23*	103.31 ± 1.15*	31.65±0.16*
Group 4: Local gin only.	157.77 ± 0.68*	149.75 ± 0.43	15.14 ± 0.67*	62.68 ± 0.08	29.95±0.09
Group 5: Palm wine +*Moringa oleifera* diet.	118.00 ± 0.56*	145.20 ± 0.89	44.13 ± 0.55*	64.83 ± 0.17	29.04±0.18
Group 6: Local gin +*Moringa oleifera* diet.	108.78 ± 0.52*	146.95 ± 1.98	40.24 ± 2.45*	59.15 ± 2.32*	29.39±0.39*

Data are represented as Mean±S.E.M. Significant with respect to the group 1 (p < 0.05) (n = 5 in each group).*

Table 3. Effects of *Moringa oleifera* on serum electrolytes in rats treated with palm wine and local gin

Groups	Sodium (mM/L)	Potassium (mM/L)	Chloride (mM/L)	Bicarbonate (mM/L)
Group 1: Positive Control.	141.48 ± 0.25	4.41 ± 0.00	100.08 ± 0.20	25.46 ± 0.02
Group 2: *Moringa oleifera* diet only.	143.41 ± 0.24*	4.81 ± 0.00*	100.02 ± 0.20	24.07 ± 0 .04*
Group 3: Palm wine only.	104.27 ± 0.21*	2.61 ± 0.02*	60.14 ± 0.07*	19.48 ± 0.01*
Group 4: Local gin only.	102.56 ± 0.22*	2.00 ± 0.01*	68.90 ± 0.11*	16.53 ± 0.02*
Group 5: Palm wine +*Moringa oleifera* diet.	140.50 ± 0.03	5.10 ± 0.03*	99.16 ± 0.11	25.52 ± 0.0 3
Group 6: Local gin +*Moringa oleifera* diet.	140.84 ± 0.02	4.41 ± 0.01	98.90 ± 0.11	25.50 ± 0.02

*All values=Mean±SEM; * significantly different from values of Group 1 at p<0.05*

Palm wine and local gin caused considerable liver damage through induction of peroxidation of lipids and finally inhibits the protein synthesis [34]. The treatment with *M. oleifera* stabilized the serum total protein and albumin levels. The stabilization of proteins might be considered as an indication of enhanced protein synthesis in the hepatic cells due to inhibition of peroxidation of lipids and scavenge of the free radicals [35]. Stabilization of serum total bilirubin level by the administration of *M. oleifera* is further a clear signal of the improvement of the functional status of the hepatic cells [36].

Earlier researchers, [37,38,39,40], have demonstrated that, active compounds like flavonoids, triterpenoids, saponins and alkaloids, which are also present in *M. oleifera* are known to possess hepatoprotective property. Treatment with *M. oleifera* enriched diet attenuated the increase in enzyme activities produced by the palm wine and local gin, and a subsequent recovery towards normalization of these enzymes strongly suggests the possibility of *M. oleifera* being able to affect the hepatocytes so as to cause accelerated regeneration of parenchymal cells and lysosomes, thus protecting against lysosomal integrity and cell membrane fragility, and therefore decreasing the leakage of marker enzymes into the circulation [41].

Elevated levels of all lipoproteins except the HDL are associated with increased risk of atherosclerosis. High level of triglycerides and LDL are associated with coronary artery disease [42]. Therefore, the reduction in serum TC concentration effected by *Moringa oleifera* diet in these rats is beneficial, and may reduce the risk of cardiovascular disease.

The LDL which is popularly known as the “bad cholesterol” is highly atherogenic [43], because they are primary carriers of plasma cholesterol which builds up slowly in the walls of arteries, feeding the heart and brain. As a result of this, it forms plaque that clots the arteries thereby causing atherosclerosis and increasing the risk of high blood pressure which may eventually lead to stroke [44].

HDL is considered to have anti-atherogenic properties, since there is negative correlation between HDL and risk of cardiovascular disease. HDL is referred to as the ‘good’ cholesterol because, it is involved in transport of cholesterol from peripheral tissues to liver and thereby reducing the amount stored in the tissue and the possibility of developing atherosclerotic plaques [43]. Increased HDL concentration in rats fed with *Moringa oleifera* diet indicates that the diet may help to increase transport of peripheral tissue cholesterol to liver and thereby decrease blood cholesterol.

Electrolytes are very important for the proper functioning of cells. Alcohol-induced diuresis, reduced subjects’ plasma volume in the work done by Noth, Swislocki and Puddey [45,46], as the diuretic effects of alcohol could lead to dehydration. In the present study, supplementation of *Moringa oleifera* in the diet fed to the rats exposed to palm wine and local gin increased the electrolytes levels as an indication of improved water and electrolytes balance. Sodium together with potassium assists in the maintenance of the body's electrolyte and water balance, also in nerve conduction, muscle contraction, and the trans-port of substances across membranes [47]. Other animal studies involving chronic alcohol intake have shown significant retention of water, sodium, potassium,

and chloride after the first week of daily alcohol ingestion [48].

5. CONCLUSION

In conclusion, this study reveals the potential of *Moringa oleifera* leaf enriched diet, in improving the toxic effects of palm wine and local gin on some biochemical parameters in albino rats.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that 'Principles of laboratory animal care" (NIH publication No 85-23 revised 1985) were followed as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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