Trad. Med. J., January-April 2022
Vol. 27(1), p 15-23
ISSN-p : 1410-5918 ISSN-e : 2406-9086

Submitted : 24-10-2021
Revised : 23-12-2021
Accepted : 21-01-2022

Serum Biochemical Changes in Alloxan-induced Diabetic Rats and Ameliorative Effects of *Moringa oleifera* and *Morinda lucida* Leaf Extracts

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ABSTRACT

This study evaluated the antidiabetic properties and biochemical changes in alloxan-induced diabetic rats treated with *Moringa oleifera* and *Morinda lucida* leaf extracts. The acute toxicity values of the extracts were determined before evaluating their antidiabetic effects in 7 groups of 4 rats each. Rats in groups 1-6 were made diabetic via a single injection of alloxan monohydrate (160 mg/kg i.p). Animals whose blood glucose levels rose to 200 mg/dl and above were considered diabetic and used for the study, but group 7 was the normal control. Groups 1 and 2 received 500 and 250 mg/kg of *M. oleifera* extract, respectively, while groups 3 and 4 received 500 and 250 mg/kg of *M. lucida* extract, respectively. Group 5 received 3 mg/kg of the standard drug (Daonil), while groups 6 and 7 were the diabetic and normal control groups, respectively. Treatment lasted for 21 days and was administered orally. The phytochemical screening results showed that each of the extracts was rich in phytochemical agents. Higher amounts of flavonoids and terpenoids were found in *M. oleifera,* while *M. lucida* had higher phenols and saponins. LD_{50} value obtained for each extract was above 5000 mg/kg. Results of the antidiabetic study indicated that the extracts significantly brought down glucose levels in the test rats ($p<0.05$) when compared with the diabetic control. Treatment also restored elevated lipids values, liver function, and renal function parameters in the diabetic rats. The decreased glutathione, catalase, and superoxide dismutase activities and increased lipid peroxidations resulting from diabetes mellitus induction were also significantly corrected following extracts administration when compared with the diabetic control ($p<0.05$). Our findings show that *Moringa oleifera* and *Morinda lucida* extracts may be safe for use in the management of diabetes mellitus and its associated biochemical complications.

Keywords: Diabetes mellitus; *Moringa oleifera*; *Morinda lucida*; antioxidants; liver functions; renal markers; lipid profile

INTRODUCTION

The use of medicinal plants as healing agents is widely accepted today and is currently recognised as an alternative means of improving global healthcare, especially in low-income countries. Factors like availability, acceptability, negligible side effects, and perceived potency are some reasons for the current global acceptance of these treatment alternatives (Orieke *et al*., 2019). Chemical substances from plants (phytochemical agents)are generally known to be responsible for the healing effects of plants and have therefore been used to protect the body against diseases and to treat diseases. This may be why plants are currently seen as key contributors to success in the ongoing search for new therapeutic agents against diseases, of which diabetes mellitus is part (Mafu and Zerbe, 2018).

Diabetes mellitus is a disorder of the endocrine usually caused by inadequate secretion of insulin or resistance. A common feature of the disease is elevated blood glucose levels leading to hyperglycemia and dyslipidemia (Maritim *et al*., 2003). Control of blood sugar through diets adjustment is a well-recognized prevention strategy, especially in the face of perceived inefficacy of currently available over-the-counter medicines. Dietary plans involving the consumption of herbs and extracts from plants are nutritional tools for use in limiting the global impact of the disease. *Moringa oleifera* and *Morinda lucida* are among medicinal plants of interest in the ongoing search for cheap, readily

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available, and effective management agents for diabetes mellitus.

Moringa oleifera (drum stick tree or "horse-riding tree) is a medicinal plant that belongs to the family *Moringaceae*. The flowers reportedly contain a stimulant that traditionally has found usefulness in the treatment of inflammatory diseases. Seeds from the plant have also been employed in the treatment of diseases of the liver and to bring down elevated blood pressure. The leaves are also used to treat hyperglycemia and diseases caused by microbial agents (Igwe *et al*., 2015). The leaves are also consumed as vegetables because of their high vitamins, antioxidants, and macronutrients contents (Asare *et al*., 2012), even though the absence of toxicity claim on *M. oleifera* needs to be investigated further (Robinson *et al.,* 2008, Gasi, 2000). On the other hand, *Morinda lucida* (family *rubiaceae*) is a tropical West Africa rainforest tree. The tree is called sangogo in Cote d'Ivoire; in Ghana, it is called ewe amake, while in Togo, the name is atakake. The Yorubas of southwest Nigeria call it cruwo Nigeria (Dalziel, 1937). Varying healing effects have been attributed to different parts of the plant. For example, in Cameroon, the leaves are macerated in cold water, and the resulting solution is used to treat fever. Water decoctions of the roots, leaves, and stem bark are used to treat dysentery and stomach colic and to expel intestinal helminths (Dalziel, 1937). The Yorubas of Nigeria use the extract of the leaves in palm wine to manage diabetes mellitus. Results of scientific investigations on extracts prepared from *M. lucida* leaves showed that the extracts possess significant trypanocidal, antimalaria, and vaso-relaxant effects (Osuntokun, 2021). The effectiveness of these medicinal plants against diabetes mellitus and hyperlipidemia lipid has before now been reported and was attributed to their thiocarbamate glycosides and β-sitosterols contents (Nayak *et al*., 2020; Yassa and Tohamy, 2014). In fact, *Moringa oleifera* leaf extract is known to specifically ameliorate alloxan-induced diabetes in rats via mechanisms such as β cells regeneration and pyruvate carboxylase expression reduction (Abd El Latif *et al*., 2014). These established antidiabetic activities of *Moringa oleifera* and *Morinda lucida* may be the reason for their preference over other herbs in diabetes mellitus management.

It was against this background that in this study, the effects of *M. lucida* and *M.oleifera* leaves extracts on serum biochemical changes were investigated in alloxan-induced diabetic rats.

METHODOLOGY

Collection and identification of plant materials

M. oleifera and *M.lucida* leaves were collected from a botanical garden at the Michael Okpara University of Agriculture Umudike, Ikwuano Local Government Area of Abia State, and were authenticated by a plant scientist at the Department of Plant Science and Biotechnology of the same University. Dried samples of the plant materials were assigned voucher numbers MOUAU/ZEB/18/005 for *M.oleifera* and MOUAU/ZEB/18/006 for *M. lucida* before being preserved at a herbarium at the University.

Preparation of plant extract

The extract was prepared from each plant material following a standard stepwise procedure. First, the leaves were dried under the shed and pulverized. A known quantity of each powdered material was soaked in ethanol and macerated. For *M. oleifera,* 250 g of material was soaked in 1 liter of ethanol. The same was done on *M.lucida* in a separate container. Each plant material in ethanol solution was vigorously steered at intervals for 48 hours before filtration to obtain filtrate containing the extract in the solution. These solutions were concentrated to dryness at a low temperature (40 ^{0}C) in an oven to obtain pasty extracts of *M. oleifera* and *M. lucida* with percentage yields of 5.52% and 5.97%, respectively. The extracts were preserved in the refrigerator until use and are hereafter referred to as MOLE for *M. oleifera* leaf extract and MLLE for *M. lucida* leaf extract

Quantitative phytochemical evaluation of the extracts

Quantitative phytochemical analyses were carried out following standard protocols. Alkaloids, flavonoids, and steroids were determined following the methods described by Haborne (1998). Saponin was determined following AOAC (2000) methods. Cardiac glycosides were quantified following the techniques described by Onwuka (2005). Phenol and tannins were determined using the methods described by Ejikeme *et al*. (2014).

Experimental Animals

Twenty-eight adult male and female Wistar albino rats (120-150 g) obtained from Ogive Integrated Farms Ltd Abayi, Osisioma Local Government Area of Abia State, Nigeria, were used for the study. The animals were divided into seven groups of 4 rats, and each group was housed in

Table I. Quantitative phytochemical composition of the MOLE and MLLE

Parameter (mg/100 g)	*M.oleifera*	*M.lucida*
Alkaloids	1.30±0.14	0.85±0.08
Flavonoids	21.23±2.87	7.04±1.07
Saponins	12.72±2.31	16.89±2.33
Tanins	0.22±0.06	0.29±0.04
Phenols	0.16±0.02	7.69±0.98
Cardiacglycosides	1.32±0.13	1.08±0.05
Terpenoids	11.51±1.12	5.75±0.54

Values are means ± SEM for N = 3.

separate aluminium cages and were exposed to standard environmental conditions with access to food and water *ad libitum* but were denied food for 12 hours before beginning the experiment. Experiments were carried out following international guidelines relating to the use and care of laboratory animals (OECD, 2008).

Induction of diabetes and experimental design

The animals in all groups (except group 7) were made diabetic by a dose administration of alloxan monohydrate (160 mg/kg b.w, i.p) and were confirmed to be diabetic in a further five days via the determination of the glucose concentration of blood collected from each rat by tail snip using an Accu-check active glucometer and test strips. Animals with blood sugar levels above 180 mg/dl were considered diabetic and used. The groups were treated according to the order: group 1 (MLLE, 250 mg/kg), group 2 (MLLE, 500 mg/kg), group 3 (MOLE, 250 mg/kg), group 4 (MOLE, 500 mg/kg), group 5 (Daonil, 3 mg/kg), group 6 (untreated diabetic rats ie negative control) and group 7 (normal control). Treatment for the rats was oral and twice daily (morning and evening) except for the standard drug group, which was once daily. After 14 days of treatment, the rats were sacrificed by cervical dislocation, and blood was collected by cardiac puncture into plain bottles and allowed to clot within one hour before centrifugation at 3000 rpm for 10 minutes to collect sera properly labeled plain bottles. Biochemical tests were carried out on the collected sera.

Biochemical analysis

Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin, total protein, urea, creatinine, albumin, serum potassium, serum sodium, and lipid profile parameters including total cholesterol, high-density lipoprotein, and triglycerides were all determined using commercial test kits produced by Randox laboratories, U.K. Catalase (CAT) activity, Malondialdehyde (MDA) concentration, superoxide dismutase (SOD) and glutathione (GSH)activities were all determined following the methods described by Misra and Fridovich (1972).

Statistical analysis

Data generated from the study were analyzed using the statistical package SPSS version 22.0. Group comparisons were made using the analysis of variance (ANOVA) test. Significant differences between control and experimental were assessed by the least significant difference (LSD). All data were expressed as mean ± SEM. P-values less than 0.05 were considered to be significant.

RESULT AND DISCUSSION

Result

Quantitative phytochemical composition of the extracts

Significant amounts of alkaloids, flavonoids, saponins, tannins, phenols, cardiac, and terpenoids were found in both *M. oleifera* and *M. lucida* leaf extracts. However, *M. oleifera* extracts contained higher amounts of alkaloids, flavonoids, cardiac glycosides, and terpenoids, while saponins and phenols were found in significantly higher amounts in *M. lucida* extract (Table I).

Effects of MOLE and MLLE on fasting blood glucose levels of diabetic rats

When compared with the diabetic untreated rats ($p<0.05$), both MOLE and MLLE significantly lowered glucose levels in the test rats by the end of treatment. The glucose-lowering effects of the extracts were also compared favorably with that of the standard drug used (Table II).

Table II. Effects of MOLE and MLLE on fasting blood sugar levels of diabetic rats

Groups	Treatments (mg/kg bw)	Initial glucose conc. (mg/dl)	Finial glucose conc. (mg/dl)
1	Diabetic + 250 MLLE	208.51±5.53	72.31±5.19*
2	Diabetic + 500 MLLE	272.03±4.98	72.38±3.38*
3	Diabetic + 250 MOLE	264.08±10.12	73.69±4.76*
4	Diabetic + 500 MOLE	291.55±8.65	100.61± 3.82*
5	Diabetes + 0.3 Daonil	208.59±7.65	76.62±4.22*
6	Diabetic control	292.07±9.54	203.63±8.79*
7	Control	95.06±5.90	97.08±5.09*

*Values are means ± SEM for N = 4. Values of final glucose concentration marked * are significantly different from their corresponding initial values at p< 0.05.*

Table III. Effect of MOLE and MLLE on biochemical values of diabetic rats

Treatment (mg/kg)	AST (iu/L)	ALT (iu/L)	ALP (iu/L)	T.Protein (g/dl)	T.BIL (mg/dl)	Urea (mg/dl)
Diabetic + 250 MLLE	37.00±0.58*	30.67±1.86*	12.60±0.31	4.50±0.10*	1.03±0.38*	27.50±1.63*
Diabetic + 500 MLLE	36.67±1.20*	30.00±1.53*	13.27±0.28	4.77±0.03*	1.33±0.18*	31.13±1.63*
Diabetic + 250 MOLE	36.33±2.03*	29.00±2.52*	13.07±0.12	6.00±0.32*	1.63±0.12	32.30±0.78*
Diabetic + 250 MOLE	35.00±2.52*	29.00±0.58*	12.63±0.32	7.83±0.19*	1.40±0.12*	33.17±0.58*
Standard drug	33.67±1.86*	28.33±1.20*	12.93±0.47	4.27±0.22*	1.53±0.03*	24.80±1.20*
Diabetic control	84.00±1.53	52.00±2.52	13.50±0.55	3.80±0.10	1.97±0.03[c]	45.93±1.65
Normal control	30.00±0.58*	20.67±1.20*	10.30±0.15*	6.90±0.15*	0.63±0.03*	23.80±0.45*

*Values are means ± SEM for N =4. Values in the same column marked * are significantly different from that of the diabetic control group at p< 0.05.*

Effects of MOLE and MLLE on some biochemical parameters of diabetic rats

Serum biochemical parameters including AST, ALT, total bilirubin, and urea were significantly higher in the diabetic animals when compared with the normal control group ($p<0.05$). However, treatment with the extracts significantly lowered the values of these biochemical parameters by the end of treatment ($p<0.05$). The lowered total protein values in the untreated rats were also significantly raised following treatment ($p<0.05$), but ALP concentrations were not significantly altered (Table III).

Effects of MOLE and MLLE on the lipid profile of diabetic rats

No significant change was observed between the total cholesterol values of the diabetic untreated rats and the ones treated with both MOLE and MLLE after 21 days ($P>0.05$). However, significantly elevated triglycerides and low-density lipoprotein cholesterol concentrations were significantly lowered following treatment ($p<0.05$). Low HDL-C values were also significantly increased by the end of the treatment period ($p<0.05$) and restored to levels that compared favorably with normal values in rats (Table IV).

Effects of MOLE and MLLE on serum antioxidant activity

Glutathione and catalase activities were significantly increased following treatment with the extracts ($p<0.05$), but the increased lipid peroxidation (MDA) observed in the diabetic untreated group was lowered significantly in groups treated with MOLE and MLLE. The activities of SOD were not significantly altered across the groups ($p>0.05$). These results are presented in Table V.

Table IV. Effect of MOLE and MLLE on the lipid profile of diabetic rats

Groups	Treatments (mg/kg)	CHOL (mmol/L)	TAG (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)
1	Diabetic + 250 MLLE	3.46±0.16	1.38±0.08*	1.77±0.09*	1.48±0.17*
2	Diabetic + 500 MLLE	3.69±0.20	1.62±0.06*	1.73±0.09*	1.62±0.12*
3	Diabetic + 250 MOLE	3.17±0.06	1.40±0.18*	1.67±0.03*	1.33±0.01*
4	Diabetic + 500 MOLE	3.36±0.14	1.62±0.04*	1.50±0.10*	1.28±0.07*
5	Standard drug	3.85±0.22	1.70±0.07*	1.73±0.03*	1.78±0.22
6	Diabetic control	3.68±0.06	1.92±0.04	1.43±0.03	1.90±0.08
7	Normal control	3.67±0.14	1.52±0.05*	1.77±0.09*	1.56±0.20*

*Values are means ± SEM for N =4. Values in the same column marked * are significantly different from that of the diabetic control group at p< 0.05.*

Table V. Effect of MOLE and MLLE on serum antioxidant enzyme levels

Treatments (mg/kg)	GSH (u/dl)	MDA (mmol/L)	Catalase (u/dl)	SOD (u/dl)
Diabetic + 250 MLLE	8.80±0.10*	1.57±0.18*	0.98±0.05	10.75±0.27
Diabetic + 500 MLLE	7.52±0.20*	1.82±0.04	1.12±0.06*	10.63±0.61
Diabetic + 250 MOLE	9.79±0.11*	1.33±0.05*	1.18±0.03*	11.14±0.23
Diabetic + 500 MOLE	11.28±0.21*	0.75±0.03*	1.18±0.02*	10.96±0.15
Standard drug	5.21±0.09*	1.59±0.13*	0.88±0.05	11.29±0.17
Diabetic control	4.79±0.12	1.86±0.07	0.92±0.06	11.05±0.06
Normal control	6.41±0.33*	1.37±0.04*	0.87±0.06	11.15±0.17

*Values are means ± SEM for N = 4. Values same column marked * are significantly different from that of the diabetic control group at p< 0.05.*

Discussion

The fact that diabetes Mellitus is a chronic disorder of carbohydrate, fat, and protein metabolism characterized by increased fasting and postprandial blood sugar levels is well established (Joseph *et al.,*2011). Alloxan induces the disease by precipitating a redox cycle which causes the generation of superoxide radicals that selectively destroys pancreatic β-cells and cause failure or total absence of insulin synthesis and secretion (Sollu *et al*., 2010), the effect of which is sustained hyperglycemia (Akomas *et al*., 2014). Medicinal plants with hypoglycemic activities may increase insulin secretion by enabling glucose utilization by the peripheral tissues and inhibiting its absorption from the intestine and production from the liver (Hui*et al.,* 2009). The fact that acute toxicity values for *M. oleifera* and *M. lucida* were both reported to be well above 6000 mg/kg body weights in rats (Hisham*et al*., 2015; Agbo*et al*., 2012) suggest that these medicinal plants may be safe for use as food and in the management of diseases. The degree of antidiabetic activity produced by the extracts in this study may be due to their significantly high flavonoids, alkaloids, tannins, phenols, and saponin contents. Flavonoids and phenols are specifically known for their strong antioxidant potentials, and antioxidant agents are known to be of value in the treatment of oxidative stress-related diseases like diabetes mellitus (Oshilonyah *et al.*, 2015). The values of flavonoids and phenols in treating this disease have also been reported (Olajide *et al*., 1999). This may be why in this study, the extracts were able to significantly counter the oxidative effects of alloxan in the diabetic rats, and the process produced significant ameliorative effects. The observed hypoglycaemic effect in the diabetic rats following treatment with the extracts attributes antidiabetic property to the extracts, which may be via inhibition of the activities of enzymes that are responsible for the degradation of oligosaccharides and disaccharides into absorbable monosaccharide (glucose) in the small intestinal brush border, leading to eventual fall in blood sugar levels. Similar conclusions were reached in a related study that evaluated the antidiabetic effect of *Mucuna pruriens*, a common medicinal plant in the southeast, Nigeria (Akomas *et al*., 2014). The extracts may have also achieved these antihyperglycaemic effects by enhancing insulin secretion from residual pancreatic beta cells and increasing peripheral use of glucose (Akomas *et al*., 2014b).

The high serum lipids values in the diabetic rats may be due to an increase in the mobilization of free fatty acids from peripheral fat deposits into the systemic circulation (Bays *et al*., 2004). Hyperlipidemia is an established cause of complications in diabetes mellitus (Akomas *et al*., 2014). The lowering of total cholesterol, triglycerides, and LDL-Cholesterol in the extract-treated suggests that the extracts may contain bioactive substances with hypolipidaemic effects. There is sufficient literature data to support the hypolipidaemic and anti-hyperlipidaemic effects of phytochemical agents like tannins, flavonoids, and saponins (Ezekwe and Obidoa, 2001; Nwanjo, 2005); therefore, the presence of these phyto-agents in *M.oleifera* and *M.lucida* may be responsible for the observed effects. In diabetes mellitus, high levels of triglycerides, LDL-C, and VLDL-C have been associated with cardiovascular traumas and insulin resistance usually accompanying the condition (Pushparaj*et al.,* 2000). The fact that at all doses of both extracts reduced serum concentrations of cholesterol, triglycerides, LDL-C, and VLDL-C and increased that of HDL-cholesterol suggests that the extracts may be of value in the control diabetic hyperlipidemia. These findings agree with the reports of Ramesh (2015) and Owoade *et al.* (2017), who before now reported the anti-hyperlipidaemic activities of *M. oleifera and M. lucida,* respectively, in diabetic rats.

Oxidative stress is known to be partly responsible for the onset of diabetes mellitus and its associated complications due to the increased generation of reactive oxygen species (ROS), which characterize the condition (Giacco and Brownlee, 2010). Oxidative stress in diabetes mellitus may be due to glucose protein glycation, auto-oxidation, and the polyol pathway (Rains and Jain, 2011). It is now established that a high concentration of blood glucose impairs or depresses the natural antioxidant defense system, thus promoting oxidative stress and the eventual onset of diabetes mellitus (Gumieniczek*et al.,* 2002). Therefore, restoring the body's antioxidant defense line may be of value in the prevention of diabetes mellitus and its complications. In this study, leaf extracts of *M.oleifera* and *M.lucida* successfully increased the values of antioxidant parameters like glutathione and catalase and reduced lipid peroxidation activities in the diabetic rats, suggesting that both extracts may be rich in antioxidant phytochemicals. The antioxidant activities of flavonoids, phenols, and tannins already reported in this study may be the reason for this activity of the extracts. The roles of flavonoids and phenols in enhancing the body's antioxidant strength have been reported (Obogwu *et al*., 2014)

Findings in this study that liver enzymes activities were significantly increased in the diabetic rats are consistent with literature data (Iweala and Oludare, 2011). Liver damage is usually assessed by the serum levels of ALT and AST (Dobbs *et al*., 2003). High levels of AST and ALT indicate liver damage, cardiac infarction, and muscle injury. However, ALT is more specific to the liver and is thus a better parameter for detecting liver injury. Serum ALP, on the other hand, is related to the function of hepatic cells and biliary obstruction. High values of AST, ALT and ALP have been reported following alloxan-induced diabetes in rats (Johnson *et al.,* 2014). This increase is mainly due to the leakage of these enzymes from the liver cytosol into the bloodstream (Johnson *et al.,* 2014). The lowering effect of the extracts on the activities of these enzymes is, therefore, suggestive of their hepatoprotective potentials (Nwanjo, 2007). The values of other liver function parameters after treatment further support the reported hepatoprotective effects of the extracts (Tuvemo*et al*., 1997; Bakris, 2007; Luke *et al.,* 2013) and may be linked to their flavonoids and phenolic compounds content. These phytochemical agents protect liver cells by exerting membrane-stabilizing effects on the liver cells (Khileifat*et al.,* 2002).

The kidney regulates the re-absorption of electrolytes into blood (Day and Mayne, 1994), but when its function is compromised, substances like chloride and potassium that are normally cleared accumulate in the biological fluid (Liamis *et al.,* 2013). The above normal levels of chloride (hyperchloremia) and potassium (hyperkalemia) observed in the diabetic control group suggests that the normal excretion of these electrolytes by the kidneys was adversely affected. Furthermore, the decrease in the levels of sodium and bicarbonate in the diabetic control group suggests that some aspects of tubular functioning as it relates to these electrolytes have been compromised. The bicarbonate ion maintains a healthy acidity level in the blood and other fluids in the body. The amelioration observed in groups treated with *moringa* and standard drugs suggests that these agents may possess some level of nephroprotective activities. This effect has indeed been reported for the aqueous extract and oil of *M. oleifera* seed (Muhammad *et al.,* 2016) and other medicinal plants *(*Dineshkumar*et al.,* 2010).

CONCLUSION

The findings of this study support the reported use of the leaf extracts of *M. lucida* and *M. oleifera* in the management of diabetes. Extracts from these plants have shown significant anti-hyperglycemic, anti-hyperlipidaemic and antioxidant potentials. The possible mechanism of action could be the antioxidant effects of their phytochemical components.

CONFLICT OF INTEREST

None declared.

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