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Evaluation of the Anti-Diabetic and Antioxidant Activities of Aqueous Extracts of *Morinda lucida* and *Saccharum officinarum* Leaves in Alloxan-Induced Diabetic Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Authors OO and TO designed the study and wrote the protocol. Author OO managed the literature searches, performed the statistical analysis, and wrote the first draft of the manuscript. Authors OO, CM and SA prepared the plant materials and performed the laboratory analyses. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aim: To investigate the antidiabetic, anti-hyperlipidaemic, and antioxidant potentials of the combined aqueous extracts of *Morinda lucida* and *Saccharum officinarum* leaves.
Study Design: Thirty alloxan-induced diabetic male rats were randomly and evenly distributed into six groups, and were subsequently exposed to the following treatments for twenty-one days: Group I (Control): Normal saline; Group II: Untreated Diabetic control; Group III: Diabetic rats treated with glibenclamide (600mg/Kg. b.w); Group IV: Diabetic rats treated with *Morinda lucida* (400mg/Kg b.w); Group V: Diabetic rats treated with *Saccharum officinarum* (400mg/Kg b.w); Group VI: Diabetic group treated with *Morinda lucida* and *Saccharum officinarum* (400mg/Kg b.w, 1:1).
Place and Duration of Study: This work was carried out in the Department of

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Biochemistry, University of Lagos, Lagos, Nigeria between November 2012 and February 2013.

Methodology: Blood samples were collected for the determination of fasting blood sugar and biochemical profiles following the last oral treatment and an overnight fast. The pancreas, liver, and kidney tissues of each animal were excised and subjected to tests for oxidative stress markers.

Result: There was a significant reduction ($P< 0.001$) in the fasting blood glucose of diabetic rats treated with the plant extracts, both separately and the extract mixture when compared to the untreated diabetic group. Diabetic treated groups showed a significant decrease *(P<0.001)* in the levels of Total cholesterol and Low Density Lipoprotein Cholesterol when compared to the diabetic untreated group. Levels of reduced glutathione and Catalase activities in the pancreas and liver of diabetic treated groups were significantly increased compared to the untreated diabetic control *(P<0.001)*. Activities of Super-Oxide Dismutase were significantly increased *(P<0.001)* in the pancreas and kidney of rats treated with the plant extracts while Malondialdehyde showed a significant reduction in the treated groups of all organs evaluated *(P<0.001)*.

Conclusion: The extracts showed anti-hyperglycaemic, anti-hyperlipidaemic and antioxidant potentials.

Keywords: Alloxan-induced diabetes; oxidative stress; dyslipidaemia; Morinda lucida; Saccharum officinarum.

1. INTRODUCTION

Diabetes mellitus is a severe health problem, a heterogenous metabolic disorder characterised by defects in insulin secretion, insulin action, or both [1]. The incidence is on the increase all over the world in both developing and developed countries predisposing chronic complications like retinopathy, nephropathy, coronary heart disease, and neurologic disease [2]. The global prevalence of diabetes is estimated to increase, from 4% in 1995 to 5.4% by the year 2025 [3].

Numerous studies have demonstrated that oxidative stress, mediated mainly by hyperglycaemia-induced generation of free radicals, contributes to the development and progression of diabetes and related complications [4]. Diabetes causes disturbances of lipid profiles, much more causing increasing susceptibility of cell membrane to lipid peroxidation [5]. Antioxidants may offer resistance against oxidative stress by scavenging the free radicals, inhibiting the lipid peroxidation [6]. By extension therefore, combating effects of oxidative stress through treatment with natural compounds that possess potent antioxidant activities might be an effective strategy for reducing diabetic complications.

Traditional systems of medicine is widely practised today on many accounts which include population rise, inadequate supply of drugs, prohibitive cost of treatments, side effects of several allopathic drugs. These have led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments [7]**.**

Morinda lucida Benth (Rubiaceae) is a medium-sized tree, about 15m tall (lucifera). It is a tropical West Africa rainforest tree popularly known as Brimstone tree [8]. It has different local names among different West Africa countries where the plant is found. In Nigeria, the plant is widely used in the treatment of typhoid fever, jaundice, and malaria [9].

Phytochemical constituents of aqueous and methanolic extracts of *M. lucida* stem bark have shown the presence of flavonoids, saponins, glycoycosides and steroids [8] while the methanolic extract of *M. lucida* leaves contain flavonoids, alkaloids, tannins and saponin [10].

Saccharum officinarum, family Poaceae, is generally called sugarcane while it is known as Ireke among the indigenous people of South Western Nigeria.

Among the traditional practitioners in Lagos, Nigeria, fresh leaves of *M. lucida* and *S. officinarum* are squeezed, mixed with water and taken orally in the treatment of diabetes mellitus. Hence, this study aimed at evaluating the folkloric use of the plants in the management of diabetes.

2. MATERIALS AND METHODS

2.1 Plant Materials and Preparation of Extracts

Fresh leaves of *M. lucida* benth and *S. officinarum* were collected from Ilaro, Ogun State, Nigeria in November 2012. The plants were identified and authenticated by Mr. Banji Oyetola, a taxonomist in the herbarium, Department of Botany, University of Lagos, Akoka, Lagos. The voucher numbers were LUH 3819 and LUH 5516 for *M. lucida* benth and *S. officinarum* respectively. The leaves of *M. lucida* and *S. officinarum* were air dried in an open space of laboratory. Aqueous infusions of the plants were prepared to mimick the native traditional method of preparation by performing a cold maceration of 200 g each of the pulverized plant material in 1000 mL of distilled water. It was kept in the Laboratory at room temperature for 48 hours after which it was filtered using muslin cloth and filter paper. The filtrate was collected in a beaker; the water was allowed to evaporate over a water bath to yield 19.6g and 15.4g extract concentrate of *M. lucida* and *S. officinarum* respectively. The concentrates were dissolved in normal saline for this study.

2.2 Experimental Animals

Twenty wistar rats, twelve weeks old weighing (120-150g) and thirty Swiss albino mice (weighing 18.85± 0.27 g) were obtained from the animal house College of Medicine of the University of Lagos, Idi-Araba. The animals were housed in clean plastic cages, well ventilated (26 ± 2ºC and relative humidity 30-35%) in 12 hours light and 12 hours dark cycle respectively. The animals were provided with standard pelleted rat chow and water ad libitum, and were allowed to acclimatize for two weeks before commencing the experiment. This research was conducted in accordance with the internationally accepted principles for laboratory animal use and care (NIH Publication: 83-23) [11] and in strict compliance to the guidelines of the Ethical committee on the use and care of animals of the College of Medicine, University of Lagos.

2.2.1 Acute toxicity study

The toxicity study was carried out using thirty (30) Swiss albino mice of both sexes. The animals were randomly grouped into six groups (five in each group) and treated with oral administration of plant extracts as follows after fasting them for 16 hours: Group I received normal saline (control); Group II received 100mg/Kg of *M. lucida*; Group III received 1000mg/Kg of *M. lucida*; Group IV received 2000mg/Kg of *M. lucida;* Group V received

1000mg/Kg of *S. officinarum* and Group VI received 2000mg/Kg of *S. officinarum*. The animals were observed continuously for the first 4 hours and then each hour for the next 24 hours; and at 6 hours intervals for the next 48 hours after the administration of the respective extracts to observe any death, external body surface changes or changes in general behaviour of the animals [12,13].

2.2.2 Induction of diabetes in rats

Thirty Wistar male rats weighing 139.30 ± 5.69 were induced with diabetes by intraperitoneal injection of alloxan monohydrate ($C_4H_2N_2O_4.H_2O$, 10%), 150mg/kg body weight dissolved in normal saline (0.2 ml, 154mM Nacl) after 18 hours fasting to induce hyperglycaemia in experimental rats [14]. The blood glucose level was monitored for 72 hours after alloxan administration in blood samples collected by tail tipping method using a Glucometer (ACCU-CHEK, Roche Diagnostics). Animals with blood glucose level greater than 250mg/dl were considered diabetic and selected for this study.

2.3 Experimental Design

Aqueous leaf extracts of *M. lucida* and *S. officinarum* were administered through oral route as follows:

Group I: (Control): Normal saline
Group II: Diabetic control untreated
Group III: Diabetic rats treated with glibenclamide (600mg/Kg. b.w)
Group IV: Diabetic rats treated with *M. lucida* (400mg/Kg b.w)
Group V: Diabetic rats treated with *S. officinarum* (400mg/Kg b.w)
Group VI: Diabetic group treated with *M. lucida* and *S. officinarum* (400mg/Kg b.w, 1:1).

Following twenty-one days of treatment, blood samples were collected through cardiac puncture into Lithium heparin bottles for biochemical analysis after an overnight fasting. Pancreas, kidney and liver were excised, blotted with tissue paper and suspended in cold normal saline for rinsing. The blood samples were centrifuged at 3500 rpm for 10 minutes. The plasma was used for the biochemical assay.

2.3.1 Tissue sample preparation

The pancreas, liver, and kidney tissues excised from each animal were homogenised in cold buffer using teflon homogeniser in a tissue to buffer ratio of 1:10. The resulting tissue buffer mixture was centrifuged at 3500 rpm for 10 minutes at 4℃ and the supernatants were kept at -20^{0}C and were assayed within 48 hours for markers of oxidative stress.

2.4 Assessment of Biochemical Parameters

Aspartate Aminotransferase-AST and Alanine Aminotransferase-ALT were both determined by the method of Reitman and Frankel, [15] using Randox Reagent Kits. Determination of Alkaline Phosphatase was done using Teco Reagent kit according to the method described by Tietz et al. [16]. Biolabo reagents were used for the determination of Urea [17], Creatinine [18], Triglycerides (GPO method) by Fossati et al. [19], Total Cholesterol by Allain et al. [20], High Density Lipoprotein (HDL) – Cholesterol (PTA) by Tietz [21]. LDL-Cholesterol was determined mathematically using the following formula as posited by Lopez et al. [22]:

LDL–Cholesterol = Total Cholesterol – (TG/2.2) – HDL-Cholesterol.

2.5 Evaluation of Oxidative Stress Markers

2.5.1 Determination of superoxide dismutase (SOD) activity

Superoxide dismutase (EC 1.15.1.1) catalyses the of superoxide anions converting them to hydrogen peroxides. Superoxide Dismutase activity was determined by its ability to inhibit the auto-oxidation of epinephrine determined by the increase in absorbance at 480nm as described by Sun and Zigma [23]. The reaction mixture (3 ml) contained 2.95 ml 0.05 M sodium carbonate buffer pH 10.2, 0.02 ml of liver homogenate and 0.03 ml of epinephrine in 0.005 N HCL was used to initiate the reaction. The reference cuvette contained 2.95 ml buffer, 0.03 ml of substrate (epinephrine) and 0.02 ml of water. Enzyme activity was calculated by measuring the change in absorbance at 480 nm for 5 mins.

2.5.2 Catalase activity determination

Catalase (EC 1.11.1.6) activities of liver and brain tissues were determined by Goth's colorimetric method [24], in which homogenate was incubated with H_2O_2 substrate. The enzyme reaction was stopped by the addition of ammonium molybdate. The intensity of the yellow complex formed by molybdate and H_2O_2 was measured at 405nm.

2.5.3 Reduced glutathione determination

The reduced glutathione (GSH) content was estimated according to the method described by Sedlak and Lindsay [25]. To the homogenate 10% TCA was added, centrifuged. 1.0ml of supernatant was treated with 0.5ml of Ellmans reagent (19.8mg of 5,5-dithiobisnitro benzoic acid (DTNB) in 100ml of 0.1% sodium nitrate) and 3.0ml of phosphate buffer (0.2M, pH 8.0). The absorbance was read at 412nm.

2.5.4 Lipid peroxidation

Malondialdehyde (MDA), an index of lipid peroxidation was determined using the method of Buege and Aust [26]. 1.0 ml of the supernatant was added to 2 ml of (1:1:1 ratio) TCA-TBA-HCl reagent (thiobarbituric acid 0.37%, 0.24N HCl and 15% TCA) tricarboxylic acid-thiobarbituric acid-hydrochloric acid reagent boiled at 100^{o}C for 15 min, and allowed to cool. Flocculent materials were removed by centrifuging at 3000 rpm for 10 minutes. The supernatant was removed and the absorbance read at 532 nm against a blank. MDA was calculated using the molar extinction coefficient for MDA-TBA complex of 1.56×10^5 $M^{-1}CM^{-1}$.

2.5.5 Determination of total protein

This was determined using Biuret method [27]. 5.0 ml of blank Biuret reagent prepared by dissolving $CuSO_{4..}5H_20$ crystal in 500 ml of distilled water was added to sample blank. These were mixed well and allowed to stand for 20 min at room temperature 25 - 27^{o}C. Absorbance was read for one test and standard against a blank at 540 nm. The concentration of protein was calculated using: optical density for standard × concentration of standard.

2.6 Statistical Analysis

Data were analyzed by comparing values for different treatment groups with the values for individual controls. Results were expressed as mean ± SEM. The significant differences among values were analysed using analysis of variance (One-way ANOVA) and Bonferroni for post hoc tests (multiple comparison) at *P-value* = 0.05. Graph pad prism 5.0 software was used for the analysis.

3. RESULTS

Acute toxicity study of the two extracts did not show any sign of mortality or toxicity in the animals indicating that the extracts are safe at the administered dosage (2000mg/Kg b. wt) and non-toxic to the animals.

Table 1 showed the blood glucose level in the control and experimental groups. There was no significant difference in the basal blood glucose in the test groups and the control group. After the three days of diabetes induction with alloxan monohydrate, a significant rise in fasting blood glucose was recorded and after twenty-one days of plant extracts administration, there was a significant reduction (*P<0.*001) in the blood glucose of diabetic rats treated with the plant extracts, both separately and the extract mixture compared to the diabetic control rats. No significant difference was observed in the blood glucose of diabetic rats treated with the standard drug, glibenclamide and the plant extracts treated groups though diabetic rats administered with *S. officinarum* and those treated with the combined *M. lucida* and *S. officinarum* showed lower fasting blood glucose levels.

The groups administered with the plant extracts showed a significant reduction *(P<0.001)* in the levels of AST, ALT, and creatinine when compared to the diabetic control group *(P<0.001)*. The rats administered with combined plant extract mixture had a significant increase in HDL when compared to the diabetic control whereas the *M. lucida* administered group was not significant. The plant extract groups except those administered with *S. officinarum* only also showed a significant reduction in the levels of ALP, Urea, and TG when compared with the diabetic control group *(P<0.001)*. The lipid profile of diabetic treated groups showed a significant decrease *(P<0.001)* in the levels of Total cholesterol and LDL-Cholesterol when compared to the diabetic untreated group (Table 2).

Table 3 showed the effects of extracts on the antioxidant enzymes. Evaluation of reduced glutathione and Catalase activities in the pancreas and liver of diabetic treated groups were significantly increased compared to the non-treated diabetic control (*P<0*.001). Levels of Super-Oxide Dismutase were significantly increased (*P<0*.001) in the pancreas and kidney of rats treated with the plant extracts while malondialdehyde showed a significant reduction in the treated groups of all organs evaluated (*P<0*.001).

Table 1. Blood glucose level (mg/dl) variation during the 21 days treatment

Groups	Group I	Group II	Group III	Group IV	Group V	Group VI
Basal Value	83.6±3.5	80.8±5.6	70.6±6.9	70.4±7.5	91. 0±2.7	80.0±4.9
After Induction	83.4±3.7	431.8±51.8 [a]	421.8±47.6 [a]	516.6±10.2 [a]	381.6±42.6 [a]	444.6 ±44.4 [a]
After Treatment	85.0±2.7	389.6±40.2 [a]	120.6±5.9 [b]	123.0±9.4 [b]	110.2±3.0 [b]	110±3.5 [b]

Values are expressed as Mean ± SEM (n=5); Statistical significance at P< 0.001

a: significant compared with control; b: significant compared with diabetic group; c: significant compared with group III. Group I - Non-diabetic Control; Group II – Diabetic control; Group III – Diabetic + Glibenclamide; Group IV – Diabetic + M. lucida; Group V – Diabetic + S. officinarum; Group VI – Diabetic + M. lucida + S. officinarum.

Table 2. Biochemical profiles of experimental rats after 21 days of treatment

Parameters	Group I	Group II	Group III	Group IV	Group V	Group VI
AST (U/L)	110.2±4.09	273.8±19.67 [a]	159.0±15.02 [ab]	126.0 ±1.62 [b]	107.5±3.71 [b]	62.84±0.94 [abc]
ALT (U/L)	24.52±0.72	108.4 ± 4.91 [a]	69.7 ± 0.46 [ab]	71.68 ± 2.16 [ab]	100.0 ± 3.53 [abc]	14.68± 0.55 [a, b]
ALP	60.50±0.45	151.20±2.17 [a]	136.20 ± 1.94 [ab]	92.51 ± 1.59 [ab]	145.30 ± 3.21 [a]	1.25±0.01 [abc]
Urea (mmol/L)	2.81 ± 0.36 [b]	12.04 ± 0.41 [a]	11.21 ± 0.08 [a]	8.93 ± 0.05 [ab]	12.00 ± 0.68 [a]	2.68 ± 0.19 [bc]
CREA (mmol/L)	15.58 ± 0.16 [b]	90.68 ± 0.49 [a]	81.88 ± 0.70 [ab]	81.04 ± 0.58 [ab]	62.40 ± 2.65 [a,b]	15.28± 0.18 [b]
T-CHOL (mmol/L)	2.77 ± 0.03	6.49 ± 0.14 [a]	4.38 ± 0.09 [ab]	3.79 ± 0.11 [ab]	4.27 ± 0.02 [a, b]	3.31±0.05 [abc]
TG (mmol/L)	0.72 ± 0.02	2.38 ± 0.03 [a]	1.38 ± 0.04 [ab]	1.30 ± 0.05 [ab]	2.34 ± 0.06 [a]	1.78±0.01 [abc]
HDL (mmol/L)	1.53 ± 0.08	0.43 ± 0.01 [a]	1.08 ± 0.04 [ab]	0.42 ± 0.01 [a]	0.81± 0.01 [a, b]	1.77±0.02 [abc]
LDL (mmol/L)	0.91 ± 0.01	4.76±0.19 [ab]	2.64 ± 0.01 [ab]	2.76 ± 0.01 [ab]	2.37 ± 0.01 [ab]	0.72±0.01 [b]

Values are expressed as Mean ± SEM (n=5); Statistical significance at P< 0.001

a: significant compared with control; b: significant compared with diabetic group; c: significant compared with group III. Group I - Non-diabetic Control; Group II – Diabetic control; Group III – Diabetic + Glibenclamide; Group IV – Diabetic + M. lucida; Group V – Diabetic + S. officinarum; Group VI – Diabetic + M. lucida + S. officinarum.

Table 3. Effects of the extracts on antioxidant enzymes

	Organ	Group I	Group II	Group III	Group IV	Group V	Group VI
GSH	Pancreas	1.56±0.02	0.91±0.04 [a]	1.15±0.01 [ab]	1.37±0.02 [ab]	1.17±0.02 [ab]	1.41 ± 0.01 [ab]
	Kidney	1.33±0.01	0.77±0.05 [a]	1.07 ± 0.02 [a]	1.17±0.01	1.00±0.05 [a]	1.16 ± 0.02 [a]
	Liver	1.25±0.01	0.51±0.01 [a]	0.81±0.01 [ab]	1.54±0.01 [ab]	0.66±0.01 [ab]	1.21 ± 0.01 [bc]
SOD	Pancreas	2.40±0.01	0.56±0.01 [a,]	1.51±0.01 [ab]	1.13±0.12 [ab]	1.04±0.02 [a, b]	1.20 ± 0.01 [ab]
	Kidney	1.82±0.01	0.32±0.02 [a]	0.98±0.01 [ab]	1.17±0.01 [ab]	1.77±0.13 [b]	1.04±0.05 [ab]
	Liver	1.74±0.01	0.74±0.01 [a]	1.05±0.01 [ab]	1.82±0.01 [ab]	0.80±0.01 [a]	1.23±0.01 [abc]
CAT	Pancreas	9.91±0.01	4.55±0.03 [a]	8.02±0.01 [ab]	6.40±0.20 [ab]	6.47±0.02 [ab]	6.26±0.02 [ab]
	Kidney	4.73±0.01	2.99±0.31 [a]	4.17±0.31	4.27±0.26 [b]	4.46±0.29 [b]	4.14±0.27
	Liver	8.80±0.58	1.39±0.01 [a]	1.96±0.01 [a]	5.53 ±0.06 [ab]	3.68±0.14 [ab]	3.49±0.07 [a, b, c]
MDA	Pancreas	0.11±0.00	0.87±0.01 [a]	0.17 ± 001 [ab]	0.15 ± 0.01 [ab]	0.35±0.01 [ab]	0.19±0.00 [abc]
	Kidney	0.16±0.00	0.23±0.01 [a]	0.16 ± 0.01 [b]	0.13 ± 0.00 [ab]	0.17±0.00 [b]	0.12±0.01 [ab]
	Liver	0.06±0.00	0.19±0.00 [a]	0.12 ± 0.01 [ab]	0.15 ± 0.00 [ab]	0.12±0.00 [ab]	0.11±0.00 [abc]

Values are expressed as Mean ± SEM (n=5); Statistical significance at P<0 .001

a: significant compared with control; b: significant compared with diabetic group; c: significant compared with group 3. Group I - Non-diabetic Control; Group II – Diabetic control; Group 3 – Diabetic + Glibenclamide; Group IV – Diabetic + M. lucida; Group V – Diabetic + S. officinarum; Group VI – Diabetic + M. lucida + S. officinarum.

4. DISCUSSION

Diabetes is a chronic disorder of carbohydrate, fat, and protein metabolism characterised by increased fasting and post prandial blood sugar levels [3]. Alloxan is a chemical that is generally used in experimental animals to induce diabetes by a way of establishing a redox cycle resulting in the production of superoxide radicals. A sudden selective destruction of pancreatic β-cells occurs when the radicals undergo dismutation to hydrogen peroxide with a concurrent massive increase in cytosolic calcium concentration [28].

One of the fundamental basis of hyperglycaemia in diabetic condition is the overproduction of glucose by means of excessive hepatic glycogenolysis and gluconeogenesis [29]. Medicinal plants with hypoglycaemic activities increase insulin secretion by enabling glucose utilization by the peripheral tissues and inhibit glucose absorption from intestine and glucose production from the liver [30]. Leaves extract of *M. lucida* has been reported to contain high concentrations of flavonoids, alkaloids, tannins and saponin and hypoglycaemic activities of flavonoids and alkaloids have been documented [10,31].The hypoglycaemic effect of the plant extracts shown in this study could be by a way of inhibiting enzymes in the small intestinal brush border that are responsible for the degradation of oligosaccharides and disaccharides into monosaccharide suitable for absorption. This action in turn will halt glucose entry into the systemic circulation and lower post prandial glucose concentrations [28].

Assessment of biochemical profiles of ALT, AST, and ALP are important in the evaluation of liver integrity and damage [32]. Marked increase in release of hepatic enzymes into the blood stream is often associated with massive necrosis of liver [33].

Urea is the predominant nitrogen waste product of protein catabolism; when in excess urea induces generation of free radicals and oxidative stress [34,35]. Urea and creatinine are important markers of kidney function. The significant reduction of urea and creatinine levels observed in this study suggests that the aqueous leaf extract of *M. Lucida* leaves could aid kidney recovery functions in diabetes caused kidney dysfunction.

Dyslipidaemia is a common diabetic condition sequel to both insulin deficiency and insulin resistance which affect enzymes and pathways of lipid metabolism [36]. High levels of Total Cholesterol, triglycerides, and LDL- Cholesterol in the diabetic rats observed in this study compared to the non-diabetic control group are in tandem with what has been reported that elevated serum concentrations of total cholesterol, low density lipoprotein (LDL) cholesterol and triglycerides are usually evident in diabetic conditions [37]. This study further revealed the hypolipidaemic potential of separate extract and the combined extracts in lowering the levels of TC and LDL-Cholesterol. Significant reduction in the lipid profile of the ethanolic root extract of *M. lucida* has been reported [38].

During diabetes, persistent hyperglycaemia promotes increased production of Oxygen free radicals as a result of glucose auto-oxidation, lipid peroxidation of low density lipoprotein, and in the processes involved in the formation of advanced glycation end products (AGE) [39,4]. Increase in the levels of Oxygen free radicals have been implicated in the pathophysiology of diabetes mellitus which could be due to their increased formation and/or decreased destruction by enzymic (e.g. Catalase and Super-Oxide Dismutase) and non-enzymic antioxidants (e.g. reduced glutathione) [40]. The decreased activity of antioxidants and elevated lipid peroxide levels in the diabetic untreated rats observed in this study could be linked to oxidative stress and/or decreased antioxidant defence mechanisms [41].

Impaired glutathione metabolism is also implicated in the pathogenesis of diabetes [42]. Glutathione, a substrate for glutathione peroxidase and glutathione S-transferase, is mainly concerned with the removal of oxygen free radicals and maintenance of membrane protein thiols [43]. Marked increased levels of reduced glutathione and Catalase in the pancreas and liver of diabetic treated groups observed could be associated to the free radical scavenging activities present in the plant extracts.

Superoxide Dismutase just like Catalase are important scanvengers of superoxide ion and hydrogen peroxide, protecting the cellular constituents from oxidative damage [44].

TBARS were measured as an index of malondialdehyde production. Elevated levels of MDA suggests enhanced lipid peroxidation resulting into tissue damage; decreased level of reduced glutathione is associated with enhanced lipid peroxidation [45]. Reduction in MDA levels of the organs of diabetic treated rats further emphasised the antioxidant potentials of the extracts.

5. CONCLUSION

The findings of this study support the reported use of the combined leaf extracts of *M. lucida* and *S. officinarum* in Lagos, Nigeria for the management of diabetes. The extracts showed anti-hyperglycaemic, anti-hyperlipidaemic, and antioxidant potentials. The possible mechanism of action could be the free radical scavenging activities of the metabolites present in the extracts.

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COMPETING INTERESTS

Authors declared that no competing interests exist.

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