Niger. J. Physiol. Sci. 34 (June 2019) 033-042
www.njps.com.ng

Niger. J. Physiol. Sci.

Morinda lucida Aqueous Stem Bark Extract Ameliorates Hepato-Renal Dysfunctions in Experimental Diabetes Model

Adeneye A. A.*[1], Olagunju J. A.[2,3], Babatunde O.[3], Oluyemi O.[3], Awosope O.[3], Oyekunle G.[3], Ayuba H.[3], Omoregie O.[3] and Omole O.[3]

Departments of [1]Pharmacology, Therapeutics & Toxicology, Faculty of Basic Clinical Sciences and [2]Medical Biochemistry, Faculty of Basic Medical Sciences, Lagos State University College of Medicine, 1-5 Oba Akinjobi Way, G.R.A, Ikeja, Lagos State, Nigeria. [3]Department of Biochemistry, Faculty of Science, Bowen University, Iwo, Osun State, Nigeria

Summary: Diabetes Mellitus (DM) is a leading pan-systemic endocrine disorder with attendant high morbidity and mortality owing to its deleterious effects on vital body organs caused by untreated chronic hyperglycemia, attendant oxidative stress and glycation processes. The present study is designed to investigate possible protective role and mechanism(s) of action of 125-500 mg/kg/day of *Morinda lucida* aqueous stem bark extract (MLASE) on renal and hepatic functions in alloxan-induced hyperglycemic rats for 8 days. Forty-two alloxan-induced hyperglycemiic male Wistar rats were randomly allotted to Groups II-VI and orally treated with 10 ml/kg/day distilled water, 5 mg/kg/day glibenclamide, 125 mg/kg MLASE, 250 mg/kg MLASE, and 500 mg/kg/day MLASE, respectively. Group I normal rats served as untreated control and were orally treated with 10 ml/kg of distilled water, all under same sham-handling. Blood samples were taken for measurement of fasting blood glucose, renal and hepatic function profile. Liver and kidney tissue samples were taken for determination of the activities of oxidative stress markers such as malondialdehyde (MDA), reduced glutathione (GSH), and glutathione peroxidase (GPx), catalase (CAT) and superoxidase dismutase (SOD). Results showed that intraperitoneal injection with 120 mg/kg of alloxan in cold 0.9% normal saline reliably and significantly induced a steadily sustained hyperglycemia which were ameliorated by short-term oral treatment with 125-500 mg/kg/day of MLASE, dose dependently, similar to that ameliorated by the standard antihyperglycemic drug, glibenclamide. Similarly, MLASE significantly mitigated against derangements in the measured renal and hepatic function parameters as well as oxidative stress induced by alloxan-induced hyperglycemia. In conclusion, results of this study showed the protective role of 125-500 mg/kg/day of MLASE in chronic hyperglycemia-associated renal and hepatic dysfunctions which was mediated via antioxidant and free radical scavenging activities of MLASE.

Keywords*:* Induced hyperglycemia, hepatic and renal function profile, oxidative stress markers, *Morinda lucida*.

©Physiological Society of Nigeria

**Address for correspondence*: adejuwon.adeneye@lasucom.edu.ng, adeneye2001@yahoo.com; +234-803-583-5589

Manuscript Accepted: April, 2018

INTRODUCTION

Diabetes mellitus (DM), a pan-systemic endocrine disorder which is characterized by chronic hyperglycemia, remains the most common disorder of carbohydrate, lipid and protein metabolism resulting from insulin deficiency and/or insulin action (American Diabetes Association, 2019).

Untreated or poorly controlled DM is often associated with multi-systemic complications such as such as vasculopathy, retinopathy, nephropathy, neuropathy and cardiovascular diseases (Kaneto *et al.*, 2007; American Diabetes Association, 2019). Indeed, DM is known to be the most common single cause of kidney failure, a condition known as diabetic nephropathy which affects about 20-30 % of diabetic subjects (Mehdi and Toto, 2009; Hahr and Molitch, 2015). Diabetic nephropathy is a major complication associated with poor diabetic control and a leading cause of end stage renal failure (Fonteles *et al.*, 2007; Alicic *et al.*, 2017). Similarly, type 2 DM is now estimated to be the most common cause of liver diseases (including abnormal hepatic enzyme levels, non-alcoholic fatty liver disease (NAFLD), cirrhosis, hepatocellular carcinoma, and acute liver failure) in the United States of America (Tolman *et al.*, 2007; Blendea *et al.*, 2010). Cryptogenic cirrhosis, of which diabetes is, by far, the most common cause, has become the third leading indication for liver transplantation in the U.S. (Golabi *et al.*, 2018).

Morinda lucida Benth, belonging to the Rubiaceae family, is a medium-sized tree used as a medicinal plant in West Africa (especially in Nigeria) in the local treatment of malaria and other febrile conditions, diabetes, hypertension, cerebral congestion, dysentery, stomach ache, ulcers, leprosy, and gonorrheal (Adeneye and Agbaje, 2008; Adebayo and Krettli, 2011). Different parts of the plant have been reported

to possess antimicrobial (Fakoya *et al.*, 2014). In the same vein, different extracts of different parts of *Morinda lucida* plant have been reported to elicit antihyperglycemic activities in different experimental diabetes models (Olajide *et al.*, 1999; Adeneye and Agbaje, 2008; Odutuga *et al.*, 2010; Adeneye *et al.*, 2017). Report equally has it that *Morinda lucida* is also one of the foremost medicinal plants used in the treatment of liver diseases in the Maritime region of Togo (Kpodar *et al.*, 2016). Amongst herbalists in South West Nigeria, water decoction of the stem bark of *Morinda lucida* is used in the management of diabetes mellitus and its attendant complications. Unfortunately, despite the wide application of different decoction of *Morinda lucida* in the management of diabetes and diabetes complications, there are no scientific reports to validate or refute this folkloric use. Therefore, the present study is designed at investigating the possible short-term protective potential and mechanism(s) of 125, 250 and 500 mg/kg/day of the *Morinda lucida* aqueous stem bark extract (MLASE) on both the hepatic and renal function parameters and the oxidative stress markers in their tissues in alloxan-induced hyperglycemic rats.

MATERIALS AND METHODS

Plant sample collection and preparation

Two kilograms of fresh stem bark and some fresh leaves of *Morinda lucida* were collected from an uncultivated farmland on the outskirt of Low Cost Housing Estate, Oke-Afa, Isolo, Lagos State, Nigeria in the month of April, 2014. The harvested plant materials were processed for voucher referencing as previously described by Adeneye and Agbaje (2008). The fresh stem bark peels were gently rinsed under tap water and dried under laboratory room temperature protected from direct heat and sunlight for 3 weeks. Afterwards, the dried samples were pulverized using laboratory hammer-mill in the Department of Pharmacognosy, Faculty of Pharmacy, University of Lagos, Idi-Araba, Surulere, Lagos State, Nigeria.

Aqueous extraction

Fifty gram (50 g) of the pulverized sample was boiled in 500 ml of distilled water under continuous stirring for 1 hour after which it was filtered using a piece of clean white 2-layer cotton cloth. The filtrate was then transferred to an aerated oven already preset at 40℃ and completely dried until solid residue was left behind. The procedure was repeated two more times. The solid residue (MLASE) obtained on each extraction process was kept in a water- and air-proof container in the refrigerator maintained at -4℃ until required for experimentation.

Determination of 2,2-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity of MLASE

The scavenging activities of MLASE against DPPH free radicals were estimated using the methods of Liyana-Pathiranan and Shadidi (2005). A solution of 0.135 mM DPPH (Sigma Aldrich, St. Louis, U.S.A.) in methanol was prepared and 1.0 ml of this solution was mixed with 1.0 ml of methanol containing 25-100 µg/ml of MLASE. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm. Ascorbic acid (Sigma Chemicals Co., St. Louis, U.S.A.) equally prepared at same concentration of 25-100 µg/ml was used as reference drug. The experiment was conducted in triplicate. The ability to scavenge DPPH radical was calculated by the following equation:

DPPH radical scavenging activity (%) =

$$\frac{Abs_{Control} - Abs_{Sample}}{Abs_{Control}} X100$$

where:

$Abs_{control}$ = Absorbance of DPPH radical + methanol

Abs_{sample} = Absorbance of DPPH radical + sample extract/standard

Determination of nitric oxide scavenging activities of MLASE

Nitric oxide scavenging activities of MLASE were evaluated using the methods of Sreejayan and Rao (1997). Ascorbic acid at same concentrations as that of MLASE (25-100 µg/ml) was used standard drug.

Inhibition (%) =

$$\frac{Abs_{Control} - Abs_{Sample}}{Abs_{Control}} X100$$

Determination of the Ferric Reducing Antioxidant Power of MLASE

MLASE reducing power was determined according to the method described by Oyaizu (1996) using ascorbic acid as the reference drug. Briefly described, 1 ml of increasing concentrations (25, 50, 75 and 100 µg/ml) of MLASE or ascorbic acid was added to 1 ml of distilled water and mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide. After incubation at 50°C for 20 mins, trichloroacetic acid (2.5 ml) was added and samples centrifuged at 3000 rpm for 10 minutes. 2.5 ml aliquot of the supernatant was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% $FeCl_3$ and the absorbance of the resulting mixtures were measured at 700 nm. 1ml of distilled water was added to 2.5 ml sodium phosphate buffer and 2.5 ml potassium ferricyanide in a test tube as the blank. The reducing power was expressed as ascorbic acid equivalent (AAE) per dry weight of crude extract (mM/mg crude extract).

Experimental animals

Healthy 8-10 weeks male albino Wistar rats (110-150 g) used in this study were obtained from Bayo Farms, Sango-Otta, Ogun State, Nigeria, after an ethical approval for the study was obtained. The rats were housed in polypropylene cages and handled in accordance with international principles guiding the

Use and Handling of Experimental Animals (NIH publication 85-23, 1985) and Committee for the Update of Guide for the Care and Use of Laboratory Animals (2011). Rat feed (Livestock Feeds, Lagos, Nigeria) and tap water were provided *ad libitum*. The rats were maintained at an ambient temperature between 23-26 ºC, humidity of 60 ± 5%, and 12 hour day/night photoperiod.

Experimental induction of diabetes mellitus

Experimental type 1 diabetes was induced in 50 rats using the method described by Venugopal *et al.* (1998) and as modified by Iwalewa *et al.* (2008). Rats were injected with freshly prepared 120 mg/kg body weight of alloxan monohydrates dissolved in sterile cold 0.9% normal saline, given via the intraperitoneal route. The rats were then orally treated with 5% dextrose solution for the next 24 hours in order to prevent hypoglycemia which often accompanies alloxan-associated hyperinsulinemia resulting from massive pancreatic β-cells destruction (Gupta *et al.*, 1984). Fasting blood glucose levels in rats were measured on the 3rd day post-alloxan injection and treated rats with fasting blood glucose levels equal to or above 200 mg/dl were considered diabetic and used for the study.

Body weight measurement

Body weights of all rats were measured on the 1st and 8th day of study using digital Mettler weighing balance (Mettler Toledo Type BD6000, Mettler-Toledo GmbH, Greifensee, Switzerland). The weight difference on the 1st and 8th day in reference to the initial weight per group was calculated.

Experimental design and oral treatment of alloxan-induced hyperglycemic rats

Oral treatments of alloxan-induced hyperglycemic rats for 8 days were as follows:

- Group I: normal control rats received 10 ml/kg and 1 ml/kg of distilled water via the oral and intraperitoneal routes, respectively
- Group II: alloxan-induced hyperglycemic rats received 10 ml/kg of distilled water
- Group III: alloxan-induced hyperglycemic rats orally received 5 mg/kg of glibenclamide in distilled water
- Group IV: alloxan-induced hyperglycemiic rats orally treated with 125 mg/kg of MLASE in distilled water
- Group V: alloxan-induced hyperglycemic rats orally treated with 250 mg/kg of MLASE in distilled water
- Group VI: alloxan-induced hyperglycemic rats orally treated with 500 mg/kg of MLASE in distilled water

Blood glucose measurement

Whole fasting blood glucose (FBG) of treated rats was collected by tail tipping method and determined by the glucose oxidase method of Trinder (1969) using a One Touch Basic Blood Glucose Monitoring System® (Life Scan Inc., Milpitas, California, U.S.A.). The blood glucose monitor was calibrated and validated at the beginning of, midway into and at the end of the experiment.

Measurement of hepatic function and renal function parameters

On day 8, after an overnight fast, the final fasting blood glucose was determined before treated rats were sacrificed after light diethyl anesthesia. After anesthesia, blood samples were collected directly from the heart chamber into 10 ml plain bottles. The blood samples obtained were immediately frozen at -70 ºC and centrifuged at 3000 rpm for 20 min to separate out the serum that was then analyzed for the serum electrolytes (sodium, potassium, chloride, bicarbonates), urea and creatinine using standard diagnostic test kits (Randox Laboratories, Crumlin, U.K.) on Automated Clinical System (Sychron Clinical System®, model: CX5 PRO) (Beckman Coulter Inc., Galway, Ireland). Similarly, hepatic function as measured by serum liver enzymes (AST, ALT, ALP), proteins (total protein, albumin), lipids (TG, TC, LDL-c, HDL-c) and bilirubin (total and conjugated bilirubin) were measured using standard procedures and standard diagnostic test kits (Randox Laboratories, Crumlin, U.K.) on Automated Clinical System (Sychron Clinical System®, model: CX5 PRO) (Beckman Coulter Inc., Galway, Ireland).

Hepatic and renal tissue estimation of antioxidant and free radical scavenging activities of MLASE in alloxan-induced hyperglycemic rats

Immediately the blood samples were collected, the liver and kidneys were identified and removed and briskly rinsed in ice-cold 1.14% KCl solution in order to preserve the activities of the oxidative stress markers before there were separately homogenized in 0.1 M tris-HCl buffer of pH 7.4 to give a 10% homogenate. These homogenates were used for the appropriate oxidative stress markers estimation. Superoxide dismutase (SOD) activity in the liver and kidney tissues were determined by the method of Kakkar *et al.* (1984) while that of liver MDA, catalase (CAT) and reduced glutathione (GSH) were determined by the methods of Kumar *et al*. (2010), Sinha (1972) and Rahman *et al.* (2006), respectively. Tissue glutathione peroxidase (GPx) activity was also determined using the method of Weydett and Cullen (2010).

Statistical Analysis

Results were presented as mean ± S.E.M. for body weights and % weight changes while that of FBG and serum hepatic and renal function parameters were expressed as mean ± S.E.M. of six observations. Statistical analysis was done using two-way analysis of variance followed by post-hoc test, Student-

Newman-Keuls test, on SYSTAT 10.6. Statistical significance were considered at $p<0.05$, $p<0.001$ and $p<0.0001$.

RESULTS

Extraction of MLASE

Extraction of MLASE yielded a deep brown, sticky, solid residue which is soluble in petroleum ether, methanol, ethanol, butan-1-ol and water. The calculated yield was 12.87 ± 0.41%

DPPH free radical scavenging activities of MLASE

Using DPPH, the free radical scavenging activities of MLASE and ascorbic acid (reference drug) were observed to be dose related with MLASE at its highest dose of 100 µg/ml having the most significant ($p<0.001$) free radical scavenging activity which was comparable to that of the standard drug (ascorbic acid) (Figure 1).

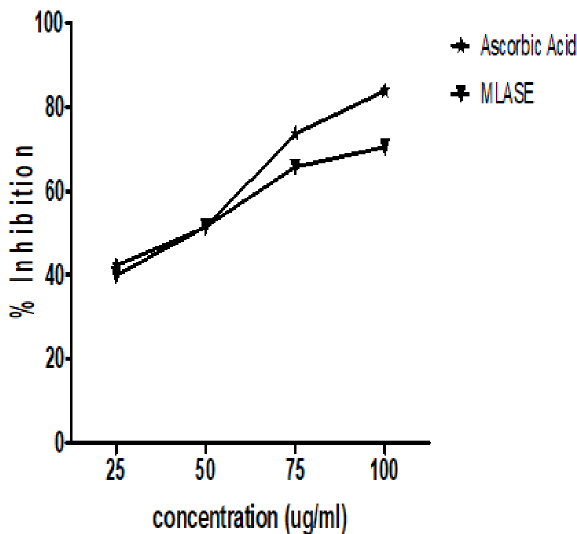

Figure 1. *In vitro* dose-related DPPH free radical scavenging activities of 25-100 µg/ml of *Morinda lucida* aqueous stem bark extract (MLASE) (test extract) and Ascorbic acid (standard drug).

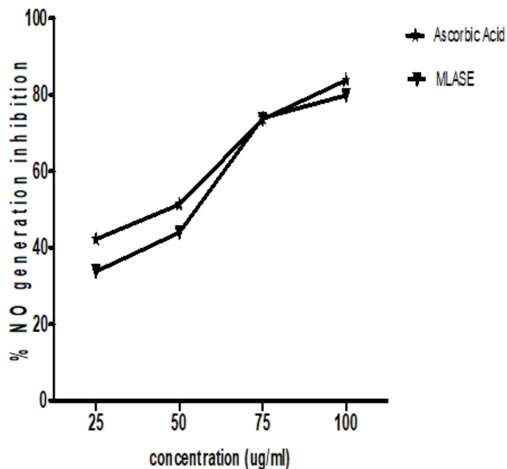

Figure 2. *In vitro* dose-related NO generation inhibitory activities of 25-100 µg/ml of *Morinda lucida* aqueous stem bark extract (MLASE) (test extract) and Ascorbic acid (standard drug)

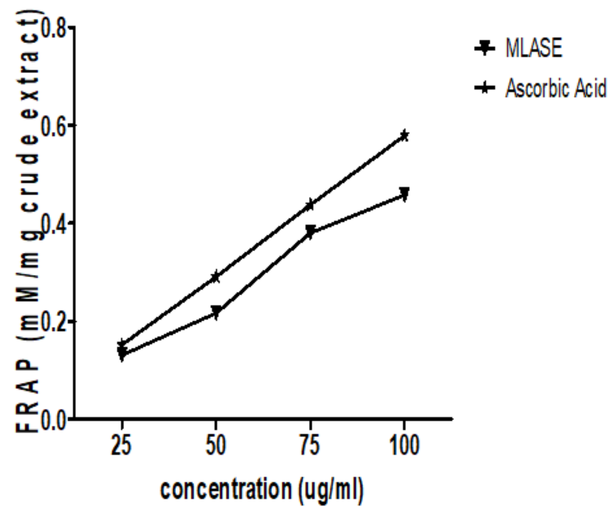

Figure 3. Ferric reducing antioxidant power of 25-100 µg/ml of *Morinda lucida* aqueous stem bark extract (MLASE) (test extract) and Ascorbic acid (standard drug)

Nitric oxide scavenging activities of MLASE

MLASE significantly ($p<0.05$) significantly inhibited the generation of nitric oxide from nitroprusside solution with the IC_{50} values of 73.98 ± 1.03 and 79.88 ± 2.00 µg/ml at 75 and 100 µg/ml, respectively which were comparative to those obtained for the standard drug (ascorbic acid) (Figure 2).

Ferric reducing antioxidant power

MLASE at concentrations (25-100 µg/ml) significantly ($p<0.05$, $p<0.01$ and $p<0.001$) inhibited the reducing power of $FeCl_3$ in a concentration-related pattern with the most significant effect recorded at the highest concentration of 100 µg/ml which favorably compared with the standard drug (ascorbic acid) at the same varying concentrations (Figure 3).

Effect of 125-500 mg/kg of MLASE on post-treatment FBG and percentage post-treatment FBG changes in alloxan-induced hyperglycemic rats

Intraperitoneal injection of 120 mg/kg of alloxan monohydrate dissolved in cold 0.9% normal saline significantly ($p<0.0001$) resulted in sustained hyperglycemia (above 200 mg/dl) by the 3rd day post-alloxan treatment (Table 1). Subsequent repeated daily oral treatments with 5 mg/kg of glibenclamide and 125, 250 and 500 mg/kg of MLASE for 8 days resulted in significant ($p<0.05$, $p<0.01$ and $p<0.001$) dose-related reductions in the FBG as well as the post-extract treatment FBG changes (Table 1).

Effect of 125-500 mg/kg of MLASE on serum renal function parameters in alloxan-induced hyperglycemic rats

Intraperitoneal injection of 120 mg/kg of alloxan monohydrate dissolved in cold 0.9% normal saline to the treated Wistar rats resulted in significant ($p<0.05$, $p<0.001$ and $p<0.0001$) increases in the serum Na^+, K^+, Cl^-, Ca^{2+}, PO_4^{2-}, uric acid, urea and creatinine when

Table 1. Effect of repeated oral treatment with 125-500 mg/kg of MLASE on the fasting blood glucose levels and percentage change in the fasting blood glucose levels in alloxan-induced hyperglycaemic rats on the 3rd day post-alloxan induction (PI) and 8th day MLASE treatment

Groups	Fasting blood glucose (FBG) levels (mg/dl) on		
	day 1	3rd day PI (with %ΔFBG)	8th day post-MLASE (with %ΔFBG)
I	51.9 ± 7.3	51.7 ± 1.6 (1.4 ± 6.2)	52.3 ± 0.9 (1.4 ± 1.9)
II	52.3 ± 1.7	228.3 ± 4.7 (336.8 ± 8.5) [c]	247.6 ± 3.0 (8.6 ± 1.4) [c]
III	59.1 ± 8.1	237.0 ± 4.8 (305.7 ± 17.2) [c]	107.1 ± 4.0 (-54.6 ± 2.2) [f]
IV	54.0 ± 5.5	231.1 ± 4.2 (331.1 ± 14.6) [c]	169.1 ± 2.2 (-26.6 ± 2.1) [e]
V	55.4 ± 10.1	228.6 ± 4.8 (323.5 ± 28.3) [c]	135.1 ± 2.52 (-40.6 ± 2.2) [f]
VI	51.1 ± 6.0	228.0 ± 5.5 (349.5 ± 10.7) [c]	101.1 ± 3.0 (-55.5 ± 2.2) [f]

[c] represents a significant increase in FBG value at $p<0.001$ when compared to FBG value on day 1 while [e] and [f] represent significant decreases in FBG values at $p<0.01$ and $p<0.001$, respectively, when compared to the 3rd day PI values and Group II values on the 8th day MLASE treatment. I = Control, II = Diabetic + 10 ml/kg of DW, III = Diabetic + 5 mg/kg of glibenclamide, IV = Diabetic + 125 mg/kg MLASE, V = Diabetic + 250 mg/kg MLASE, VI = Diabetic + 500 mg/kg MLASE

Table 2a. Effect of repeated oral treatments with 125-500 mg/kg of MLASE on serum Na^+, K^+, HCO_3^-, Cl^- , Ca^{2+} and PO_4^{2-} in alloxan-induced hyperglycaemic rats

Groups	Na^+ (mEq/L)	K^+ (mEq/L)	HCO_3^- (mEq/L)	Cl^- (mEq/L)	Ca^{2+} (mEq/L)	PO_4^{2-} (mEq/L)
I	140.1 ± 0.9	8.1 ± 1.5	20.7 ± 1.5	102.3 ± 0.8	2.2 ± 2.4	2.56 ± 0.17
II	145.4 ± 1.7[a]	17.5 ± 0.7[b]	13.1 ± 0.8[e-]	112.1 ± 0.4[c]	2.6 ± 0.1[b]	3.74 ± 0.30[a]
III	138.1 ± 1.9[e]	11.5 ± 2.9[d]	20.1 ± 1.4[b+]	100.9 ± 1.2[f]	1.9 ± 1.2[f]	2.06 ± 0.15[e]
IV	140.6 ± 0.8[d]	9.7 ± 1.4[e]	15.7 ± 1.8	103.7 ± 0.9[f]	2.3 ± 0.4[d]	3.10 ± 0.47
V	139.7 ± 1.3[d]	9.3 ± 1.1[e]	19.6 ± 1.0[b+]	103.1 ± 0.6[f]	1.8 ± 0.1[f]	2.34 ± 0.28[e]
VI	136.9 ± 0.8[e]	7.3 ± 0.7[e]	21.0 ± 0.8[c+]	99.7 ± 1.3[f]	1.6 ± 0.1[f]	2.17 ± 0.11[e]

[a, b] and [c] represent significant increases at $p<0.05$, $p<0.001$ and $p<0.0001$, respectively while [e-] represents a significant decrease at $p<0.0001$ when compared to Group I values. [e] and [f] represent significant decreases $p<0.001$ and $p<0.0001$, respectively, while [b+] and [c+] represent significant increases at $p<0.001$ and $p<0.0001$, respectively, when compared to Group II values. I = Control, II = Diabetic + 10 ml/kg of DW, III = Diabetic + 5 mg/kg of glibenclamide, IV = Diabetic + 125 mg/kg MLASE, V = Diabetic + 250 mg/kg MLASE, VI = Diabetic + 500 mg/kg MLASE

Table 2b. Effect of repeated oral treatment with 125-500 mg/kg of MLASE on serum uric acid, urea, and creatinine in alloxan-induced hyperglycaemic rats

Groups	uric acid (mg/dl)	urea (mg/dl)	creatinine (mg/dl)
I	6.2 ± 0.9	41.7 ± 1.1	1.17 ± 0.06
II	13.9 ± 0.4[b]	64.1 ± 4.8[b]	1.46 ± 0.08[a]
III	12.4 ± 1.5	47.9 ± 4.9[d]	0.70 ± 0.14[d]
IV	8.7 ± 1.1[d]	60.1 ± 5.4	1.13 ± 0.22
V	8.7 ± 1.1[d]	50.9 ± 1.8	0.97 ± 0.13
VI	6.3 ± 0.5[e]	44.1 ± 4.4[d]	0.86 ± 0.07[d]

[a] and [b] represent significant increases at $p<0.05$ and $p<0.001$, respectively when compared to Group I values while [d] and [e] represent significant decreases $p<0.05$ and $p<0.001$, respectively, when compared to Group II values. I = Control, II = Diabetic + 10 ml/kg of DW, III = Diabetic + 5 mg/kg of glibenclamide, IV = Diabetic + 125 mg/kg MLASE, V = Diabetic + 250 mg/kg MLASE, VI = Diabetic + 500 mg/kg MLASE.

compared to the values of the normal (Group I) values (Tables 2a and 2b). Alloxan treatment also resulted into a significant ($p<0.001$) decrease in the serum HCO_3^- level when compared to Group I values (Table 2a). However, with repeated daily oral treatments with 125, 250 and 500 mg/kg of MLASE for 8 days, there were significant ($p<0.05$, $p<0.001$ and $p<0.0001$) dose-related ameliorations in the serum Na^+, K^+, Cl^-, Ca^{2+}, PO_4^{2-} uric acid, urea and creatinine when compared to those values for the untreated alloxan-induced hyperglycemic rats (Tables 2a and 2b). Similarly, repeated oral treatments with 125-500 mg/kg of MLASE significantly ($p<0.001$ and

Table 3a. Effect of repeated oral treatments with 125-500 mg/kg of MLASE on serum ALB, TP, TB and CB in alloxan-induced hyperglycemic rats

Groups	ALB (mg/dl)	TP (mg/dl)	TB (mg/dl)	CB (mg/dl)
I	3.2 ± 0.1	7.1 ± 0.2	0.9 ± 0.2	0.3 ± 0.1
II	2.3 ± 0.1^{c-}	6.0 ± 0.1^{b-}	0.8 ± 0.1	0.5 ± 0.1
III	3.1 ± 0.1^{c+}	7.6 ± 0.2^{c+}	0.8 ± 0.1	0.3 ± 0.0
IV	2.8 ± 0.1^{b+}	6.3 ± 0.2	1.0 ± 0.1	0.6 ± 0.1
V	3.6 ± 0.1^{c+}	7.7 ± 0.2^{c+}	0.9 ± 0.1	0.3 ± 0.0
VI	4.2 ± 0.2^{c+}	9.2 ± 0.2^{c+}	0.8 ± 0.1	0.3 ± 0.1

$^{b-}$ and $^{c-}$ represent significant decreases at p<0.001 and p<0.0001, respectively when compared to Group I values while $^{b+}$ and $^{c+}$ represent significant increases at p<0.001 and p<0.0001, respectively, when compared to Group II values. I = Control, II = Diabetic + 10 ml/kg of DW, III = Diabetic + 5 mg/kg of glibenclamide, IV = Diabetic + 125 mg/kg MLASE, V = Diabetic + 250 mg/kg MLASE, VI = Diabetic + 500 mg/kg MLASE.

Table 3b. Effect of repeated oral treatment with 125-500 mg/kg of MLASE on serum hepatic enzymes (AST, ALT and ALP) in alloxan-induced hyperglycemic rats

Groups	AST (U/L)	ALT (U/L)	ALP (U/L)
I	109.1 ± 9.7	33.9 ± 3.7	110.6 ± 5.8
II	137.1 ± 10.6^{a}	42.1 ± 9.5^{a}	98.0 ± 7.7
III	122.4 ± 18.3^{e}	24.1 ± 2.2^{f}	84.3 ± 10.1^{d}
IV	138.9 ± 17.8^{a}	37.7 ± 4.3^{d}	90.0 ± 12.8^{d}
V	134.6 ± 13.0^{a}	27.9 ± 6.7^{f}	86.6 ± 8.6^{d}
VI	98.6 ± 9.4^{f}	23.0 ± 3.6^{f}	79.3 ± 10.8^{e}

^{a} represents a significant increase at p<0.05 when compared to Group I values while $^{d, e}$ and ^{f} represent significant decreases p<0.05, p<0.001 and p<0.0001, respectively, when compared to Group II values. I = Control, II = Diabetic + 10 ml/kg of DW, III = Diabetic + 5 mg/kg of glibenclamide, IV = Diabetic + 125 mg/kg MLASE, V = Diabetic + 250 mg/kg MLASE, VI = Diabetic + 500 mg/kg MLASE.

Table 4. Effect of repeated oral treatments with 125-500 mg/kg of MLASE on renal tissue SOD, CAT, GSH, GPx and MDA levels in alloxan-induced hyperglycemic rats

Groups	SOD (U/mg protein)	CAT (U/mg protein)	GSH (U/mg protein)	GPx (U/mg protein)	MDA (nM/mg protein)
I	74.9 ± 4.7	8.2 ± 0.7	0.4 ± 0.0	0.3 ± 0.0	0.1 ± 0.0
II	47.5 ± 5.8^{c-}	4.6 ± 0.6^{b-}	0.3 ± 0.0^{a-}	0.8 ± 0.1^{c}	0.1 ± 0.0^{b}
III	78.0 ± 6.1^{c+}	10.8 ± 1.4^{c+}	0.6 ± 0.1^{c+}	0.4 ± 0.0^{f-}	0.0 ± 0.0^{f-}
IV	76.6 ± 3.5^{c+}	8.4 ± 0.4^{b+}	0.6 ± 0.0^{b+}	0.5 ± 0.0^{f-}	0.0 ± 0.0^{f-}
V	78.7 ± 3.2^{c+}	12.5 ± 0.4^{c+}	0.6 ± 0.0^{c+}	0.4 ± 0.1^{f-}	0.0 ± 0.0^{f-}
VI	82.9 ± 1.7^{c+}	13.7 ± 0.47^{c+}	0.8 ± 0.0^{c+}	0.4 ± 0.0^{f-}	0.0 ± 0.0^{f-}

$^{a-, b-}$ and $^{c-}$ represent significant decreases at p<0.05, p<0.001 and p<0.0001, respectively while ^{b} and ^{c} represent significant increases at p<0.001 and p<0.0001, respectively when compared to Group I values. $^{b+}$ and $^{c+}$ represent significant increases at p<0.001 and p<0.0001, respectively, while $^{f-}$ represents a significant decrease at p<0.0001 when compared to Group II values. . I = Control, II = Diabetic + 10 ml/kg of DW, III = Diabetic + 5 mg/kg of glibenclamide, IV = Diabetic + 125 mg/kg MLASE, V = Diabetic + 250 mg/kg MLASE, VI = Diabetic + 500 mg/kg MLASE.

p<0.0001) restored the serum HCO_3^- to about the values recorded for normal (Group I) rats (Table 2a).

Effect of 125-500 mg/kg of MLASE on serum hepatic function parameters in alloxan-induced hyperglycemic rats

Intraperitoneal injection of 120 mg/kg of alloxan monohydrate dissolved in cold 0.9% normal saline resulted in significant (p<0.001 and p<0.0001) decreases in the serum ALB and TP while it did not cause significant (p>0.05) increases in the serum TB, CB, AST and ALT when compared to the untreated normal (Group I) values (Tables 3a and 3b). Conversely, alloxan injection did not cause significant (p>0.05) decreases in the serum ALP levels when compared to Group I values (Table 3b). However, with repeated daily oral treatments with 125-500 mg/kg of MLASE for 8 days, there were significant (p<0.001 and p<0.0001) dose-related increases in the serum

Table 5. Effect of repeated oral treatments with 125-500 mg/kg of MLASE on hepatic tissue SOD, CAT, GSH, GPx and MDA levels in alloxan-induced hyperglycemic rats

Groups	SOD (U/mg protein)	CAT (U/mg protein)	GSH (U/mg protein)	GPx (U/mg protein)	MDA (nM/mg protein)
I	63.2 ± 6.5	10.6 ± 1.2	0.4 ± 0.1	0.5 ± 0.0	0.1 ± 0.0
II	40.8 ± 1.4^{a-}	7.2 ± 0.4^{a-}	0.2 ± 0.0^{a-}	0.8 ± 0.1^{c}	0.4 ± 0.2^{c}
III	79.0 ± 6.3^{a+}	13.7 ± 1.8^{c+}	1.1 ± 0.2^{c+}	0.4 ± 0.1^{d-}	0.0 ± 0.0^{f-}
IV	76.6 ± 3.5^{c+}	8.4 ± 0.4^{b+}	0.6 ± 0.0^{b+}	0.5 ± 0.0^{d-}	0.0 ± 0.0^{f-}
V	78.7 ± 3.2^{c+}	12.5 ± 0.4^{c+}	0.6 ± 0.0^{c+}	0.4 ± 0.1^{d-}	0.0 ± 0.0^{f-}
VI	82.9 ± 1.7^{c+}	13.7 ± 0.47^{c+}	0.8 ± 0.0^{c+}	0.4 ± 0.0^{d-}	0.0 ± 0.0^{f-}

$^{a-}$ represents a significant decrease at p<0.05 while ^{c} represents a significant increase at p<0.0001 when compared to Group I values. $^{a+,\ b+}$ and $^{c+}$ represent significant increases at p<0.05, p<0.001 and p<0.0001, respectively, while $^{d-}$ and $^{f-}$ represent significant decreases at p<0.05 and p<0.0001, respectively, when compared to Group II values. I = Control, II = Diabetic + 10 ml/kg of DW, III = Diabetic + 5 mg/kg of glibenclamide, IV = Diabetic + 125 mg/kg MLASE, V = Diabetic + 250 mg/kg MLASE, VI = Diabetic + 500 mg/kg MLASE.

ALB and TP when compared to those recorded for the untreated hyperglycaemic (Group II) rats (Table 3a). However, the MLASE repeated oral treatment for 8 days did not cause significant (p>0.05) alterations in the serum TB, CB, AST, ALT and ALP levels in the Group IV-V rats when compared to the values obtained in Group II (Tables 3a and 3b).

Effect of 125-500 mg/kg of MLASE on renal tissue oxidative stress markers in alloxan-induced hyperglycemic rats

Treatment with 150 mg/kg of alloxan given intraperitoneally resulted in significant (p<0.0001, p<0.001 and p<0.05) reductions in the renal tissue SOD, CAT activities and GSH levels, respectively, while significantly (p<0.0001) enhancing the GPx and MDA activities when compared to untreated normal (Group I) (Table 4). However, oral treatments with 125-500 mg/kg/day of MLASE significantly (p<0.001 and p<0.0001) reversed these effects dose dependently with the most significant improvement recorded for the group treated with the highest dose (500 mg/kg) of MLASE when compared to untreated diabetic group (Group II) (Table 4).

Effect of 125-500 mg/kg of MLASE on hepatic tissue oxidative stress markers in alloxan-induced hyperglycemic rats

Similarly, intraperitoneal injection of 150 mg/kg of alloxan and its subsequent establishment of diabetes resulted in significant (p<0.05) reductions in the renal tissue SOD, CAT activities and GSH levels while significantly (p<0.0001 and p<0.05) enhancing the GPx and MDA activities, respectively when compared to untreated normal (Group I) (Table 5). Repeated oral treatments with 125-500 mg/kg/day of MLASE for 8 days significantly (p<0.05, p<0.001 and p<0.0001) reversed these effects dose dependently with the most significant improvement recorded for the group treated with 500 mg/kg of MLASE when compared to untreated diabetic group (Group II) (Table 5). Also, 500 mg/kg/day of MLASE offered by better ameliorative effect than that offered by the standard oral antihyperglycemic drug (10 mg/kg of glibenclamide) (Table 5).

DISCUSSION

Diabetes mellitus is a multi-systemic metabolic disorder affecting body organs such as kidney, testis, liver, heart, eyes and the brain to a varying degree depending on the severity and chronicity of the persistent hyperglycemia (Fowler, 2008; Satirapoj, 2010; Forouhi and Wareham, 2019).

The kidney is an extremely complex organ with broad ranging functions in the body, including, waste excretion, ion and water balance, maintenance of blood pressure, glucose homeostasis and generation of erythropoietin or activation of vitamin D (Forbes and Cooper, 2012; Hesaka *et al.*, 2019). With diabetes, many of these integral processes are interrupted via a combination of hemodynamic and metabolic changes; hyperglycemia also activates a series of changes leading to glomerular and tubular dysfunction and accelerates glomerular cell apoptosis (Ayodele *et al.*, 2004; Forbes and Cooper, 2012). Similarly, the liver plays an important role in the regulation of glucose, lipid and protein homeostasis, most especially, in the maintenance of blood glucose homeostasis, because it warehouses superfluous blood glucose and demobilizes same in hypoglycemic states (Indradevi *et al.*, 2012). Furthermore, the liver is the focal organ of oxidative and detoxifying processes as well as free radical reactions and the biomarkers of oxidative stress are elevated in the liver at an early stage in many diseases, including diabetes mellitus (Stadler *et al.*,

2003). In experimental diabetes, alloxan monohydrate exerts its toxic effects on kidney, liver and other organs in addition to pancreatic cells after it is accumulated intracellularly to generate cytotoxic free radicals. The pancreatic insulin insufficiency and hyperglycemia that result from pancreatic β-cell necrosis further augment renal and hepatocellular damages through reactive free radicals mediated lipid peroxidation of glomerular and hepatocellular membranes (Kume *et al.*, 2004).

In the present study, there were profound elevations in the serum levels of both hepatic and renal function parameters following the establishment of diabetes with intraperitoneal injection of alloxan monohydrate. In addition, activities of hepatic and renal tissue SOD, CAT and GSH were profoundly inhibited while those of GPx and MDA were significantly enhanced. The observations are similar to those earlier reported that oxidative stress plays a pivotal in the pathophysiology of hepatic and renal dysfunctions in the diabetic state (Albano, 2008; Kashihara *et al.*, 2010; Hahr and Molitch, 2015). Literature has it that the pathogenesis of diabetic nephropathy and hepatic dysfunction is multifactorial in which chronic hyperglycemia plays an important (Palsamy *et al.*, 2010). During diabetic milieu, supraphysiological glucose is known to result in oxidative stress from AGEs formation and the mitochondrial free radicals generation with consequent cell death, hepatic and renal dysfunctions (Forbes *et al.*, 2008). The fact that repeated oral treatments with MLASE profoundly ameliorated alterations in the hepatic and renal function and oxidative stress parameters strongly highlight the protective potential of MLASE against diabetic hepatic and renal dysfunctions. These antioxidant results of this study are strongly in consonance with the previous report of Domekouo *et al.* (2016) which reported the antioxidant properties of *Morinda lucida* aqueous stem bark extract in streptozotocin induced hyperglycemic rats orally treated with 50-500 mg/kg/day of extract for 28 days. These *in vivo* findings appeared to have been corroborated by the positive results of the *in vitro* free radicals scavenging and antioxidant studies also undertaken in the current study.

The hepatorenal protections offered by MLASE could be attributed to the presence of flavonoids, alkaloids, saponin, terpenoids, phenols, tannins and phlobatinnins in MLASE (Adeneye *et al.*, 2017) which are known to exhibit potent antioxidant activities either singly or in combination with one another as literature has shown that flavonoids, alkaloids, and other phenolic compounds to be reputably potent antioxidant phytochemicals (Rice-Evans *et al.*, 1995; Procházkova *et al.*, 2011; Orčić *et al.*, 2011). Similarly, previous other studies have attributed the antioxidant profile of *Morinda lucida* to its high phenolic contents (Ojewunmi *et al.*, 2013).

In conclusion, results of the study showed the ameliorative role of MLASE on renal and hepatic functions in the diabetic state mediated via free radical scavenging and antioxidant activities, thus, setting the scene for considering MLASE as an effective therapy in the management of not only diabetes but its associated hepatic and renal dysfunctions.

ACKNOWLEDGEMENTS

The authors express their heartfelt appreciation to Mr. Sunday Adenekan, Assistant Chief Technologist, in the Department of Biochemistry, Faculty of Basic Medical Sciences, College of Medicine, University of Lagos, Idi-Araba, Lagos State, Nigeria for his technical assistance with the oxidative stress markers assay and to Mr. M.O. Arogundade of Department of Hematology and Blood Transfusion, Faculty of Basic Medical Sciences, Lagos State University College of Medicine, Ikeja, Lagos State, Nigeria for his technical expertise with renal and hepatic function assays.

REFERENCES

Adebayo, J.O., Krettli, A.U. (2011) Potential antimalarials from Nigerian plants: A review. *Journal of Ethnopharmacology* 133(2): 289-302.

Adeneye, A.A., Agbaje, E.O. (2008) Pharmacological evaluation of oral hypoglycemic and antidiabetic effects of fresh leaves ethanol extract of *Morinda lucida* Benth. in normal and alloxan-induced diabetic rats. *African Journal of Biomedical Research* 11(1): 65-71.

Adeneye, A.A., Olagunju, J.A., Olatunji, B.H., Balogun, A.F., Akinyele, B.S., Ayodele, M.O. (2017) Modulatory effect of *Morinda lucida* aqueous stem bark extract on blood glucose and lipid profile in alloxan-induced diabetic rats. *African Journal of Biomedical Research* 20(1): 75-84.

Albano, E. (2008) Oxidative mechanisms in the pathogenesis of alcoholic liver disease. *Molecular Aspects of Medicine* 29: 9-16.

Alicic, R.Z., Rooney, M.T., Tuttle, K.R. (2017) Diabetic kidney disease: challenges, progress, and possibilities. *Clinical Journal of American Society of Nephrology* 12(12): 2032-2045

American Diabetes Association (2019) Standards of Medical Care in Diabetes - 2019. *Diabetes Care* 42(Supplement 1): S1-S2.

Ayodele, O.E., Alebiosu, C.O., Salako, B.L. (2004). Diabetic nephropathy - a review of the natural history, burden, risk factors and treatment. *Journal of the National Medical Association* 96: 1445-1454.

Blendea, M.C., Thompson, M.J., Malkani, S. (2010) Diabetes and chronic liver disease: Etiology and Pitfalls in monitoring. *Clinical Diabetes* 28(4): 139-144.

Committee for the Update of the Guide for the Care and Use of Laboratory Animals. (2011). Guide for the Care and Use of Laboratory Animal. National Academies Press, Washington DC, 8th ed.

Domekouo, U.L., Longo, F., Tarkang, P.A., *et al.* (2016) Evaluation of the antidiabetic and antioxidant properties of *Morinda lucida* stem bark extract in streptozotocin intoxicated rats. *Pakistani Journal of Pharmaceutical Sciences* 29(3):903-11

Fakoya, A., Owojuyigbe, O.S., Fakoya, S., Adeoye, S.O. (2014) Possible antimicrobial activity of *Morinda lucida* stem bark, leaf and root extracts. *African Journal of Biotechnology* 13(3): 471-5.

Fonteles, M.C., Longo, V.M., Farah, V., *et al.* (2007) Renal function alterations in *ex-vivo* perfusion in diabetic rats. *The FASEB Journal* 21: 892.10

Forbes, J.M., Cooper, M.E. (2012) Glycation in diabetic nephropathy. *Amino Acids* 42(4): 1185-1192.

Forbes, J.M., Coughlan, M.T., Cooper, M.E. (2008) Oxidative stress as a major culprit in kidney disease in diabetes. *Diabetes* 57: 1446-1454.

Forouhi, N.G., Wareham, N.J. (2019). Epidemiology of Diabetes. *Medicine* 47(1): 22-27.

Fowler, M.J. (2008) Microvascular and macrovascular complications of diabetes. *Clinical Diabetes* 26(2): 77–82.

Golabi, P., Bush, H., Stepanova, M., *et al.* (2018). Liver transplantation (LT) for cryptogenic cirrhosis (CC) and nonalcoholic steatohepatitis (NASH) cirrhosis. *Medicine* (Baltimore) 97(31): e11518

Gupta, M.P., Solis, N.G., Avella, M.E. *et al.* (1984) Hypoglycemic activity of *Neurolaena lobata*. *Journal of Ethnopharmacology* 10: 323-327.

Hahr, A.J., Molitch, M.E. (2015) Management of diabetes mellitus in patients with chronic kidney disease. *Clinical Diabetes and Endocrinology* 1: 2. doi: 10.1186/s40842-015-0001-9.

Hesaka A, Sakai S, Hamase K, *et al.* (2019). D-Serine reflects kidney function and diseases. Scientific Reports 2019; 9: Article No. 5104.

Indradevi, S., Ilavenil, S., Kaleeswaran, B., *et al.* (2012) Ethanolic extract of *Crinum ascaticum* attenuates hyperglycemia-mediated oxidative stress and protects hepatocytes in alloxan induced experimental diabetic rats. *Journal of King Saud University – Science* 24(2): 171-177.

Iwalewa, E.O., Adewale, I.O., Taiwo, B., *et al.* (2008) Effects of *Harungana madagascariensis* stem bark extract on the antioxidant markers in alloxan-induced diabetic and carrageenan-induced inflammatory disorders in rats. *Journal of Complementary & Integrative Medicine* 5(1): 1-18.

Kakkar, P., Das, B., Viswanathan, P.N. (1984) A modified spectrophotometric assay of superoxide dismutase. *Indian Journal of Biochemistry & Biophysics* 21: 130-132

Kashihara, N., Haruna, Y., Kondeti, V.K., *et al.* (2010) Oxidative stress in diabetic nephropathy. *Current Medicinal Chemistry* 17: 4256-4269.

Kaneto, H., Katakami, N., Kawamori, D., *et al.* (2007) Involvement of oxidative stress in the pathogenesis of diabetes. *Antioxidants & Redox Signal* 9: 355-366.

Kpodar, M.S., Karou, S.D., Katawa, G., *et al.* (2016). An ethnobotanical study of plants used to treat liver diseases in the maritime region of Togo. *Journal of Ethnopharmacology* 181: 263-273.

Kumar, R.P., Sujatha, D., Saleem, T.S.M., *et al.* (2010) Potential antidiabetic and antioxidant activities of *Morus indica* and *Asystasia gangetica* in alloxan-induced diabetes mellitus. *Journal of Experimental Pharmacology* 2: 29-36.

Kume, E., Fujimura, H., Matsuki, N., *et al.* (2004) Hepatic changes in the acute phase of streptozotocin (SZ)-induced diabetes in mice. *Experimental and Toxicological Pathology* 55: 467-480.

Liyana-Pathiranan, C.M., Shadidi, F. (2005) Antioxidant activity of commercial soft and hard wheat (*Triticum aestivum* L.) as affected by gastric pH conditions. *Journal of Agriculture and Food Chemistry* 53: 2433-2440

Mehdi, U., Toto, R.D. (2009) Anemia, diabetes, and chronic kidney disease. *Diabetes Care* 32(7): 1320-1326.

Odutuga, A.A., Dairo, J.O., Minari, J.B., *et al.* (2010) Anti-diabetic effect of *Morinda lucida* stem bark extracts on alloxan-induced diabetic rats. *Research Journal of Pharmacology* 4(3): 78-82.

Ojewunmi, O., Oshodi, T., Ogundele, O., *et al.* (2013) Evaluation of the antidiabetic and antioxidant activities of aqueous extracts of *Morinda lucida* and *Saccharum officinarum* leaves in alloxan-induced diabetic rats. *International Journal of Biochemistry Research & Review* 3(3): 266-277.

Olajide, O.A., Awe, S.O., Makinde, J.M., *et al.* (1999) Evaluation of the anti-diabetic property of *Morinda lucida* leaves in streptozotocin-diabetic rats. *Journal of Pharmacy & Pharmacology* 51(11): 1321-1324.

Orčić, D.Z., Mímica-Dukić, N.M., Francišković, M.M., *et al.* (2015) Antioxidant activity relationship of phenolic compounds in *Hypericum perforatum* L. *Chemistry Central Journal* 5: 34. doi:10.1186/1752-153X-5-34

Oyaizu, M. (1996) Studies on product of browning reaction prepared from glucose amine. *Japanese Journal of Nutrition* 44: 307-315.

Palsamy, P., Sivakumar, S., Subramanian, S. (2010) Resveratrol attenuates hyperglycemia-mediated oxidative stress, proinflammatory cytokines and protects hepatocytes ultrastructure in streptozotocin–nicotinamide-induced experimental diabetic rats. *Chemico-Biology Interactions* 186: 200-210.

Procházkova, D., Boušová, I., Wilhemová, N. (2011) Antioxidant and pro-oxidant properties of flavonoids. *Fitoterapia* 82(4): 513-523.

Rahman, I., Kode, A., Biswas, S.K. (2006) Assay for quantitative determination of glutathione and glutathione disulfide levels using enzymatic recycling method. *Nature Protocols* 1(6): 3159-3165

Rice-Evans, C.A., Miller, N.J., Bolwell, P.G., *et al.* (1995) The relative antioxidant activities of plant-derived polyphenolic flavonoids. *Free Radicals Research* 22: 375-383.

Satirapoj, B. (2010) Review on pathophysiology and treatment of diabetic kidney disease. *Journal of Medical Association Thailand* 93 (Suppl 6): S228-S241.

Sinha, A.K. (1972) Colorimetric assay of catalase. *Analytical Biochemistry* 47: 389-394

Sreejayan, Rao, M.N.A. (1997) Nitric oxide scavenging by circuminoids. *Journal of Pharmacy and Pharmacology* 49: 105-107

Stadler, K., Jenei, V., von Bölcsházy, G., Somogyi, A., Jakus, J. (2003) Increased nitric oxide levels as an early sign of premature aging in diabetes. *Free Radicals Biology & Medicine* 35: 1240-1251

Tolman, K.G., Fonseca, V., Dalpiaz, A., Tan, M.H. (2007) Spectrum of liver disease in type 2 diabetes and management of patients with diabetes and liver disease. *Diabetes Care* 30(3): 734-743.

Trinder, P. (1969) Determination of blood glucose using 4-aminophenzone as oxygen acceptor. *Journal Clinical Pathology* 22: 246-248.

United States National Institutes of Health. (1985) Guide for the Care and Use of Laboratory Animals. *NIH Publication* 85-23.

Weydett, C.J., Cullen, J.J. (2010) Measurement of superoxide dismutase, catalase and glutathione peroxidase in cultured cells and tissues. *Nature Protocols* 5: 51-66.

Venogopal, P.M., Prince, P.S.M., Pari, L. (1998) Hypoglycemic activity of *Syzigium cumini* seeds: effect on lipid peroxidation in alloxan diabetic rats. *Journal of Ethnopharmacology* 61: 1-7.