Evaluation of the antidiabetic and antioxidant properties of *Morinda lucida* stem bark extract in streptozotocin intoxicated rats

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Abstract: The present research evaluated the antidiabetic and antioxidant properties of *M. lucida* stem bark (50 and 500mg/kg) and glibenclamide (25mg/kg, standard drug) in acute (Oral glucose tolerance test) and sub-acute (Streptozotocin 60mg/kg, i.p. diabetic model) administration. A group of healthy rats constituted the normal control. The sub-acute experiment lasted 28 days during which water, food intake and weight gain were measured and biochemical parameters analyzed in both plasma and erythrocytes at the end of the experiment. The chemical substances present in *M. lucida* bark extract were determined. In the Oral glucose tolerance test, the reduction of blood glucose level was statistically significant for both *M. lucida* extracts and glibenclamide. However, in the diabetic rats acute administration of 500mg/kg extract had better blood sugar lowering effect than glibenclamide, which was better than 50mg/kg extract. Streptozotocin diabetic animal model was characterized by a decrease in weight gain, erythrocyte SOD and CAT activities and an increase in water and food consumption, lipid peroxidation, cholesterol, triglycerides, plasma glucose, creatinine and urea concentrations, and transaminases activities. *M. lucida* extract and glibenclamide significantly prevented the alteration of these parameters, thus indicating a corrective effect on diabetes and its complications. This study justifies the traditional claim and provides a rationale for the use of M. lucida to treat diabetes. Its antioxidant properties may serve to curb oxidative stress and hence prevent the diabetic complications related to oxidative stress. Chemical substances, which may be accountable for the antidiabetic and antioxidant properties of *M. lucida* were detected in the aqueous extract of *M. lucida* bark.

Keywords: *Morinda lucida*, streptozotocin, diabetes, antioxidant, phytochemical screening, glibenclamide, transaminases, oral glucose tolerance test.

INTRODUCTION

Diabetes mellitus is a chronic metabolic disease that remains a public health problem with 177 million diabetic patients reported in 2007 by the International Diabetes Federation (IDF) (Wens *et al*., 2007). In 2008 the World Health Organization (WHO) reported more than 180 million diabetic patients in the world (WHO, 2008). Diabetes arises from the inability of the body to produce insulin, a decrease in the response of peripheral organs to the same hormone, or both (WHO, 1994; 1999). Diabetes has been associated to an increase in oxidative stress characterized by production of oxygen-free radicals and a collapse of antioxidant defenses (Oberley, 1988). Thus, compounds that possess both hypoglycemic and antioxidant properties can serve as useful antidiabetic agents (Baynes, 1995; Soon and Tan, 2002). A number of plants / plant parts do find application in the treatment of diabetes in traditional medicine some of which include *Tecomaria capensis* Thunb. (Bignoniaceae) leaves (Saini and Singhal, 2012), *Ipomoea digitata* tuber (Pandey *et al*., 2013); *Pterocarpus marsupium* (Devgan *et al*., 2013); *Curcuma longa* (Mobasher *et al.*, 2014). *Morinda lucida* Benth (Rubiaceae) is a medium-sized tree measuring approximately 15 m in height and widely used in West and Central Africa traditional medicine. In traditional medicine, *Morinda lucida* (*M. lucida*) is used in the treatment of different types of fevers, jaundice, hypertension, cerebral congestion, dysentery, diabetes and gastric ulcer (Zimudzi and Cardon, 2005. Earlier scientific reports show that the leaves (Olajide *et al*., 1999; Adeneye and Agbaji, 2008) and the roots (Kamanyi *et al*., 1994) of *M. lucida* posses hypoglycemic/anti-diabetic activity. Activities of *M. lucida* extracts against *Salmonella typhi* (Akinyemi *et al*., 2005), isolated uterine smooth muscle contractility (Elias *et al*., 2007), toxicity and mutagenic studies (Agbor *et al*., 2012a; Sowemimo *et al*., 2007; Akinboro and Bakare, 2007; Raji *et al*., 2005), reducing and antioxidant properties (Ogunlana *et al*., 2008) have all been reported.

Phytochemical screening of *M. lucida* indicated the presence of important biological active compounds such as alkaloids, anthraquinones, anthraquinols, tannins, flavonoids and saponins (Zimudzi and Cardon, 2005). The anthraquinones have been reported to possess *in vitro*

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anti-Plasmodium falciparum activity (Koumaglo *et al*., 1992). M lucida has also been reported to possess trypanocidal (Asuzu and Chineme, 1990) and antimalarial activities (Obih *et al*., 1985). The present study tested the hypothesis that aqueous extract of *M. lucida* stem bark possesses anti-diabetic and antioxidant activity in streptozotocin intoxicated rats.

MATERIALS AND METHODS

The research carried out by applying the practice and principles of the 1996 Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act that pertains to research of this nature. This research was approved by the Ethical Review Board of the Institute of Medical Research and Medicinal Plants Studies, Yaoundé, Cameroon.

Reagents

Streptozotocin (Sigma), Glibenclamide, kits for estimation of serum glucose, cholesterol, triglyceride, creatinine and urea concentrations and transaminases activities (DIALAB diagnostic kits).

Animals

Adult albino mice (25-30g) and albino rats (150-200g) were used for this study. All animals were housed in standard conditions and received food and water ad libitum during the entire experimental period.

Preparation of plant material

The fresh stem bark of *M. lucida* was harvested at Mfou, Yaoundé Centre Region of Cameroon in the month of September by an Ethnobotanist Dr. Tsabang Nole of the Institute of Medical Research and Medicinal Plants Studies. Plant identification and voucher specimen number 2528 SRFK referencing was done at the National Herbarium, Yaoundé Cameroon. The fresh stem bark of M. lucida was cleaned and dried in an oven at low temperature (40^{o}C). The dried plant material 50g was then macerated in distilled water for 48 hours and filtered. This was repeated twice and the filtrates put together. The filtrate was then concentrated using a rotary evaporator. The concentrate was then freeze dried and stored at -20^{o}C until required.

Studies on oral glucose tolerance test (OGTT)

This test was carried out on normal as well as diabetic rats.

OGTT on normal rats

After a period of 14 hours of fasting, the experimental animals were distributed into 4 groups of 5 rats each. The animals were then administered D-glucose solution (2 g/kg). Thirty minutes later the animals were treated as follows: normal control group, distilled water (1 ml) and the standard control group, 1 ml of the reference drug (glibenclamide 0.25 mg/kg, orally). Groups 3 and 4 were administered 1 ml of *M. lucida* aqueous extract (500 and 50 mg/kg) respectively. Blood sugar concentration was then analyzed in blood samples collected from the tail vein of experimental animals before the administration of glucose (-30 min), 30 min after administration of glucose (0 min) and at 30, 60, 90 and 120 minutes after the administration of the extracts and glibenclamide using a glucometer (ONE TOUCH Ultra 2 LifeScan Inc., Johnson & Johnson Company).

OGTT on diabetic animals

The animals were made diabetic by a single dose intraperitoneal administration of streptozotocin (STZ, 60 mg/kg) prepared in NaCl (0.9%). Animals having blood glucose concentration of 250 mg/dl and above were considered diabetic and selected for the study as earlier described (Kamtchouing *et al*., 2006). Five normal (non diabetic) rats were kept aside to serve as the normal control and received distilled water while the diabetic animals were divided into 4 groups of 5 rats each. Group 2 served as diabetic control and received distilled water, group 3 standard control received reference drug, groups 4 and 5 were administered 1 ml *M. lucida* (500 and 50 mg/kg) aqueous extract respectively. Thirty minutes later the animals were administered a dose of 2g/kg of D-glucose. Blood samples collected from the tail vein of experimental animals for determination of glucose concentration before administration of glucose (-30 min) and at 0, 30, 60, 90 and 120 min after.

Antidiabetic and antioxidant activities measurement at sub-acute administration

Eight rats were kept as normal control (Group 1) while the rest of the rats (60 rats) were administered 60 mg/kg of STZ intraperitoneally to induce type 1 diabetes. The fasting blood glucose was determined 72 hours after STZ. Diabetic rats were grouped into four of eight rats each. Diabetic control (Group 2) animals were administered normal saline daily; standard control (Group 3) animals were administered glibenclamide (0.025g/kg) daily. Group 4 and 5 received the plant extract (50 and 500 mg/kg respectively) daily. This treatment schedule lasted 4 weeks during which the animals were weighed twice a week and the food consumption and water intake measured.

At the end of the experiment, blood samples were collected from the animals (under diethyl ether anesthesia) through the jugular vein into EDTA tubes. The blood was then centrifuged at 3000rpm and the plasma collected was used for the determination of plasma concentration of glucose, cholesterol, triglyceride, creatinine, urea, and aspartate aminotransferase (AST), and alanine aminotransferase (ALT) activities using the DIALAB diagnostic kits. After the removal of the plasma, the resulting red blood cells were washed thrice in NaCl (0.9%) and hemolysed by freezing Agbor *et al*., 2011, 2012b). The hemolysate was used for the analysis of

catalase (Sinha, 1972), superoxide dismutase (Misra and Fridovich, 1972), lipid peroxidation (Biswas *et al*., 1963) and total protein (Lowry *et al*., 1951).

Phytochemical screening of M. lucida stem bark aqueous extract for the presence of plants secondary metabolites

The aqueous extract of *M. lucida* was qualitatively tested for the presence of secondary metabolites using standard procedures (Trease and Evans, 1983).

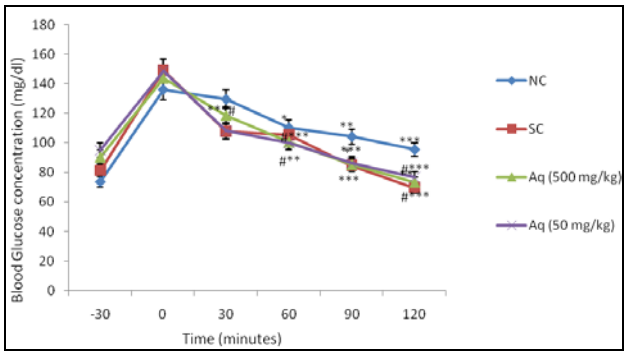
STATISTICAL ANALYSIS

Statistical analysis was carried out on results obtained. In groups' comparison One-way Analysis of Variance on Ranks was employed. The Holm-Sidak Method was used to determine significant differences between groups for all pair wise multiple comparisons. The Sigma Stat (Systat software, Richmond, CA) version 3.01 was employed in these analyses.

RESULTS

Effect on OGTT in normal animals

Effect of *M. lucida* extract on glucose tolerance test in normal animals is presented in fig. 1. The blood glucose concentration increased significantly ($p<0.05$) in the first 30 min after glucose intake. This then decreased ($p<0.001$) in all the groups from 30 min to 120 minutes (when treatment commenced). Though the standard and extract significantly ($p<0.05$) decreased the blood glucose, there was no significant difference between the effect of the aqueous extracts and the standard drug glibenclamide.

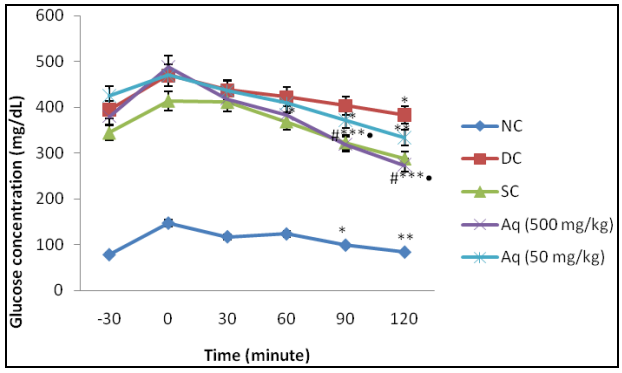


NC= Normal control, SC= standard control (Glibenclamide), Aq=aqueous extract. Each point represents Mean ± SD. * (P<0.05), ** (P<0.01), *** (P<0.001), = significant difference compared to time 0 # (P<0.05), significant difference compared to NC

Fig. 1: Effect of *M. lucida* extracts on blood glucose of normal rats

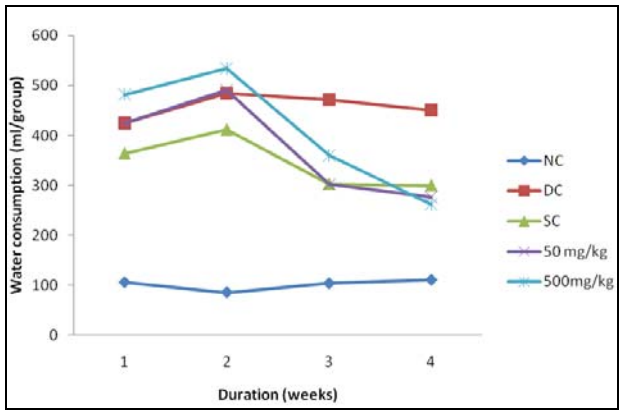
Effect on OGTT in diabetic animals

Streptozotocin significantly increased blood glucose in all groups treated. A significant reduction in blood glucose was observed in all the groups tested (fig. 2). The reduction was time dependent in the experimental groups. The reduction was observed at the 30th minute ($p<0.05$) in groups treated with 500mg/kg plant extract, at the 60th minute in standard control rats ($p<0.05$) and 90th minutes in the groups treated with 50mg/kg. The 500mg/kg extract had a better though not significant glucose lowering activity than glibenclamide. The glucose lowering effect of the 500mg/kg extract was significantly ($p<0.001$) better than the 50mg/kg extract at the 90th and 120th min of the experiment. Hence a dose dependent effect.



NC= normal control, DC= diabetic control, SC= standard control (Glibenclamide), Aq= aqueous extract. Each point represents Mean ± SD. * (P<0.05), ** (P<0.01), *** (P<0.001), significantly different compared to time 0 # (P<0.05), significantly different compared to NC • (P<0.05), significantly different compared to DC

Fig. 2: Effect of *M lucida* on blood glucose of diabetic rats.



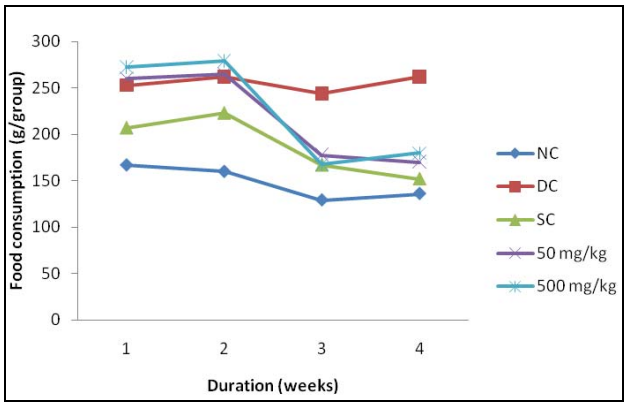
The different points represent the average water consumption in the different groups per week, n = 8.

Fig. 3: Effect of *M. lucida* on water consumption (ml/group) of diabetic rats

Effect of M. lucida on weight gain of experimental rats

Table 1 presents the effect of *M. lucida* aqueous extract on weight gain of diabetic rats. It was observed that all the groups had significant ($p<0.001$) increase in weight from week zero to week four. Considering the percentage weight gain by each group of animals, the normal control

had the highest percentage weight gain than the diabetic groups of animals. Meanwhile the percentage weight gain of the standard control and the *M. lucida* treated groups were higher than the diabetic control. In the same manner the groups treated with *M. lucida* extract had higher weight gain than the glibenclamide treated group. Hence diabetes induced weight loss and the extract of *M. lucida* prevented the effect of diabetes.



The different points represent the average water consumption in the different groups per week, n = 8

Fig. 4: Effect of *M. lucida* on food (g/group) consumption of diabetic rats.

Effect of M. lucida on water and food consumption of diabetic rats

The effect of *M. lucida* aqueous extract on water consumption is presented in fig. 3. The normal control rats presented the lowest and relatively constant volume of water consumed throughout the experimental period compared to the groups induced with diabetes. The diabetic rats treated with glibenclamide and *M. lucida* extract had an increase in water consumption in the second week of the experimentation and then dropped lower than the diabetic control animals at the third and fourth week of experimentation. On the contrary the diabetic control rats had a relatively constant increase in volume of water consumption throughout the experimentation.

Just as in water intake, diabetes induced an increase in food consumption in experimental animals (fig. 4). This increase was inhibited in experimental animals treated with glibenclamide (standard control group) meanwhile the extract of M. lucida only had an effect at the third and fourth week of experimentation.

Effect of M. lucida on plasma biochemical parameter of experimental rats

The effect of *M. lucida* on plasma biochemical parameter of experimental rats is presented in table 2. Induction of diabetes resulted to a significant ($p<0.001$) increase in the triglyceride concentration as seen in the diabetic control animals compared to the normal control animals. Treatment with glibenclamide and *M. lucida* extract significantly reduced the triglyceride concentration compared to the diabetic group ($p<0.001$) and the normal control group ($p<0.01$). The *M. lucida* extract (500 mg/kg) had a better triglyceride lowering effect compared to the glibenclamide treated group. Cholesterol concentration significantly ($p<0.001$) increased in diabetic control rats. Administration of glibenclamide and *M. lucida* (500mg/kg) extract significantly ($p<0.001$) prevented this increase comparatively. Similar results were obtained in urea and creatinine, whose plasma concentrations increased significantly in the diabetic control rats up to 53.04% (urea) and 14.13% (creatinine) compared to the normal control. However, administration of glibenclamide significantly reduced the creatinine ($p<0.05$) and urea ($p<0.001$) concentration by 10.24% and 62.55% respectively. *M. lucida* equally significantly reduced the creatinine ($p<0.001$) and urea ($p<0.001$ for 500 mg/kg and $p<0.05$ for 50mg/kg) concentration by 15.37% and 19.43% respectively. *M. lucida* extract was more effective in reducing the creatinine and urea concentration than the glibenclamide. Induction of diabetes induced a significant ($p<0.001$) increase in the activity of AST and ALT as seen in the diabetic control. After four weeks of experimentation, glibenclamide induced a significant ($p<0.001$) reduction of the activity of AST by 46.43% and 500mg/kg extract significantly ($p<0.001$) reduced the activity of AST by 62.08% compared to the diabetic control. The effect of the 50mg/kg *M. lucida* extract administration was comparable to that of the standard control group. Glibenclamide and plant extract did not have any significant ($p>0.05$) effect on the increased activity of ALT.

Effect of M. lucida on erythrocyte antioxidant enzyme and lipid per oxidation of experimental rats

The effect of *M. lucida* on erythrocyte antioxidant enzyme and lipid peroxidation of streptozotocin intoxicated rats is presented in table 3. Intoxication with streptozotocin induced a collapse in the activity of the erythrocyte antioxidant enzymes (SOA, CAT) and increased lipid peroxidation (MDA) ($p<0.001$). Administration of *M. lucida* extract (500mg/kg) significantly ($p<0.05$) prevented the effect of streptozotocin on SOD by 33.65% as compared to the diabetic control. The extract had a better antioxidant activity than the glibenclamide reference drug. A similar situation was observed in the CAT activity where administration of glibenclamide or *M. lucida* extract improved on the erythrocyte antioxidant enzyme activity. Accompanying the decrease on the antioxidant defense was an increase in lipid peroxidation as measured by malondialdehide (MDA). Glibenclamide and *M. lucida* significantly ($p<0.05$) reduced the lipid peroxidation towards normal. The percentage reduction of lipid peroxidation was in the order of 47.78% for glibenclamide and 63.38% for *M. lucida* (50mg/kg) and 45% for *M. lucida* (500mg/kg) extract.

Table 1: Effect of *M. lucida* on percentage (%) weight gain (g) of diabetic rats

Groups	Week 0	Week 4	% Weight gain
Normal control	159.20±4.27	221.80±11.03	28.22 ± 5.08
Diabetic control	149.60±4.27	159.40±4.33	5.78 ± 0.74*
Standard control	161.60±7.26	193.80±9.17	13.57 ± 3.61$^{\#*}$
M. lucida (50 mg/kg)	148.23±4.30	182.56±5.24	18.80± 6.52$^{a\#*}$
M. lucida (500 mg/kg)	144.80±2.58	180.80 ±2.49	19.91 ± 2.37$^{a\#*}$

Results are presented as Mean ± SD, n=8 *significantly lower than normal control (P<0.001), $^{\#}$significantly different compared to diabetic control (P<0.001), ^{a}significantly different compared to diabetic control (P<0.05).

Table 2: Effect of *M. lucida* extract on plasma biochemical parameters of streptozotocin intoxicated rats

Parameters	Control	Diabetic control	Standard control	M. lucida 50mg/kg	M. lucida 500mg/kg
Glucose (mg/dl)	62.77±3.24	344.7±5.22**	114.25±4.06$^{**\#\#}$	150.23±6.31$^{b**\#\#}$	144.64±5.293$^{b\#\#**}$
Triglyceride (mg/dl)	42.12±3.58	60.91±8.78*	37.27±6.85$^{\#\#}$	25.91±4.40$^{\#\#*}$	30.63±3.73$^{\#\#*}$
Cholesterol (mg/dl)	43.84±4.24	70.85±6.88**	48.70±7.28$^{\#\#}$	53.91± 4.35$^{\#\#}$	49.04±8.70$^{\#\#}$
Urea (mg/dl)	50.37±2.51	102.15±4.17**	79.67±2.10$^{\#\#}$	38.43±2.36$^{b**\#\#}$	40.63 ±3.29$^{b**\#\#}$
Creatinine (mg/dl)	1.58 ± 0.03	1.84±0.05*	1.65±0.04$^{\#}$	1.57±0.03$^{a\#}$	1.55±0.04$^{a\#\#}$
AST (U/l)	39.59±4.79	83.79±8.28**	44.89±7.32$^{\#\#}$	46.34±5.28$^{\#\#}$	31.77±6.23$^{\#\#}$
ALT (U/l)	32.22±3.40	69.29±7.34**	63.77±10.97**	67.82±4.33**	63.08±11.05**

Results are presented as mean ± SD, n=8 ^{a}(P<0.05), ^{b}(P<0.001) = significantly different from standard control (glibenclamide) * (P<0.01), ** (P<0.001) = significantly different compared to normal control, $^{\#}$ (P<0.05), $^{\#\#}$ (P<0.001) = significantly different compared to diabetic control

Secondary metabolites detected in the M. lucida aqueous bark extract

The aqueous extract of *M. lucida* stem bark extract indicated the presence of plant secondary metabolites such as flavonoids, phenols, reducing compounds, saponnins and tannins (table 4).

DISCUSSION

Streptozotocin is a 2-deoxy-2-(3-(methyl-3-nitrosoureido)-D-glucopyranose) synthesized by Streptomycetes achromogenes (Szkudelski *et al*., 2001) which serves as a standard drug for experimental diabetes in rodents and also functions as an anti-insulinoma therapeutic agent in humans (Nesovic *et al*., 1992; Kalender *et al*., 2002). Streptozotocin selectively destroys the β-cells of the pancreas and renders it less active in the production of insulin resulting in a diabetic state (Szkudelski *et al*., 2001). We here report on the effect of *M. lucida* stem bark extract in the management of diabetic condition and its complications induced by the administration of streptozotocin. Glibenclamide was used in this study as a standard reference drug for the management of diabetes because it stimulates insulin release from β-cells and inhibits glucagon secretion (Bedoya *et al*., 1996).

The OGTT is an important measurement to judge the body's ability to consume glucose. It monitors the rate of decrease in blood glucose and gives information on how effective a medication may be in case of acute hyperglycemia. OGTT adds to fasting plasma glucose concentration to simplify and facilitate the diagnosis of diabetes. The 500mg/kg dose of aqueous extract of *M. lucida* stem bark was more effective than 50 mg/kg and not different from the glibenclamide (reference drug) treated groups in OGTT of normal and diabetic rats.

An early study by Olajide *et al*. (1999) reported the hypoglycemic and anti-hyperglycemic properties of the methanolic extract (400mg/kg) of *M. lucida* leaves in which they obtained a percentage glucose reduction of 36.94% at 4 hours and 40.65% at 12 hours. The study of Olagide *et al*. (1999) was confirmed by Adeneye and Agbaji (2008) who studied the hypoglycemic and antioxidant activity of fresh ethanolic leaves extract of *M. lucida* in normal and alloxan induced diabetic rats. In our study 500mg/kg *M. lucida* stem bark aqueous extract lowered the blood glucose with a percentage glucose reduction of 23.16% at 2 hours i.e. the time taken for the glucose concentration to return to normal. Thus, both leaves and bark of *M. lucida* possess hypoglycemic activity.

Changes in experimental animal body weight, food consumption and water intake have earlier been reported as important parameters for the pathophysiology of streptozotocin-induced diabetes (Tian *et al*., 2010). In the present study, streptozotocin administration inhibited percentage weight gain, and induced increase in water and food consumption as compared to the normal control animals. Similar results have earlier been reported (Tsubone *et al*., 2005; Jensen *et al*., 2006). However, administration of *M. lucida* and glibenclamide were able

Table 3: Effect of *M. lucida* extract on erythrocyte antioxidant enzyme and lipid peroxidation of streptozotocin intoxicated rats.

Parameters	Normal control	Diabetic control	Standard control	*M. lucida* (50mg/kg)	*M. lucida* (500mg/kg)
SOD (units/mg protein/min)	86.62±2.96	58.54±1.88**	69.24±2.29*$^{\#}$	65.41±1.28*$^{\#}$	88.24±3.27$^{bc\#}$
CAT (units/mg protein/min)	12.96±4.07	3.65±0.76**	5.87±0.54**$^{\#}$	4.83±0.82**$^{\#}$	5.67±0.79**$^{\#}$
MDA (nM)	1.70±0.17	5.98±0.40**	3.12±0.30*$^{\#}$	2.19±0.23*$^{\#}$	3.29±0.29*$^{\#}$

Results are presented as mean ± SD, n=8 *(P<0.05), **(P<0.001) =significantly different compared to normal control, $^{\#}$ (P<0.05), significantly different compared to diabetic control, ^{b}(P<0.001) =significantly different from standard control (glibenclamide), ^{c}(P<0.001) = significantly different from *M. lucida* 50mg/kg

to reverse the effect of streptozotocin by maintaining these parameters towards normal. The 500mg/kg extract was better than both glibenclamide and 50mg/kg extract.

In sub-acute administration (28 days treatment) of aqueous extract of M. lucida stem bark (500mg/kg) to diabetic rats (streptozotocin induced), plasma fasting blood glucose was significantly reduced. However, glibenclamide, the reference drug had a better glucose lowering effect. This is an indication that both plant extract and glibenclamide may stimulate insulin secretion from the remnant or regenerated β-cells (Periyar *et al*., 2009). Earlier studies on glibenclamide state that it is not effective when the β-cells are completely destroyed (Cetto *et al*., 2000; Hosseinzadeh *et al*., 2002). Thus, it is more effective in moderate diabetic than in severe diabetic situations. In the present study plasma glucose of 344.70 ±5.22mg/dl can be considered as moderate diabetes.

Hyperlipidemia contributes to major risk factors of cardiovascular diseases (Umesh *et al*., 2005). Streptozotocin-induced diabetes has been shown to increase plasma cholesterol and triglyceride concentrations (Murali *et al*., 2002; Dzeufiet *et al*., 2007). Similar results were obtained in the present study. Dyslipidemia may be induced by insulin deficiency or insulin resistance since fatty acid and triglyceride synthesis in adipose tissue and liver are regulated by insulin. At normal state the lipolytic hormones effect on the peripheral fat depots are activated by insulin resulting to triglycerides hydrolyses and prevention of free fatty acids mobilization (Briones *et al*., 1984; Nikkila, 1984). In diabetic conditions, the liver conversion of free fatty acids into phospholipids and cholesterol destined to be dis charged into blood is inhibited by insulin deficiency inactivation of the lipoprotein lipase (Shirwaikar *et al*., 2005; Pushparaj *et al*., 2007). Though insulin concentration was not analyzed in this study, both the plant extract and glibenclamide significantly prevented the increase in cholesterol and triglyceride concentrations (hyperlipidemia), thus preventing the secondary effect of diabetes.

Plasma creatinine and urea are markers of renal function. Though these metabolites are end products of protein metabolism, their concentrations remain fairly constant under normal conditions unless renal function changes (Whitby *et al*., 1988). Urea concentration may also increase in congestive cardiac failure and gastrointestinal hemorrhage (Whitby *et al*., 1988). The significant increases in the plasma urea and creatinine levels in the present study is an indication of impaired renal function in diabetic animals as earlier reported (Dzeufiet *et al*., 2007; Umila and Goyal, 2003; Periyar *et al*., 2014). Thus, M. lucida extract and glibenclamide protects against impaired renal function by inhibiting changes in plasma creatinine and urea concentrations.

Table 4: Chemical components tested in the *M. lucida* stem bark aqueous extract

Chemical components	Aqueous extract
Tannins	+
Phenols	+
Reducing substances	+
Saponins	+
Fatty acids	-
Glycosides	+
alkaloids	-
Flavonoids	+

+ = Present, - = absent

Transaminases (ALT and AST) are important hepatic markers and their presence in the plasma at high concentration serves as indicators for liver pathology. This is the case in the administration of toxins to experimental animals where there is a significant increase in the plasma level of these enzymes (Nadeem *et al*., 1997; Singh *et al*., 1998). Increase in the activities of ALT and AST in streptozotocin intoxication have earlier been reported (Nguenguim *et al*., 2007; Mobasher *et al*., 2014; Periyar *et al*., 2014). Similar results were obtained in the present study. However, both glibenclamide and *M. lucida* extracts were able to inhibit changes in ALT and AST activity in experimental animals showing their protective potentials.

Diabetes has been related to oxidative stress either as a causative factor or because of its consequence (Qia *et al*., 2008). STZ-induced diabetes is characterized not only by impaired glucose tolerance and hyperglycemia but also by a collapse in antioxidant defense mechanism. Oxidative

stress has been shown to be responsible, at least in part, for tissue damage and β-cell dysfunction (Kröncke *et al*., 1995). Reactive oxygen species produced in streptozotocin administration, which may lead to DNA fragmentation and other deleterious changes in the cells (Takasu *et al*., 1991; Bedoya *et al*., 1996). In the present study the administration of streptozotocin induced a collapse in antioxidant defense as observed in a decrease in SOD and CAT activities and an increase in lipid per oxidation product (MDA) in the diabetic control group. This implies marked oxidative stress in animals intoxicated with streptozotocin in accordance with earlier results (Soon and Tan 2002; Saxena *et al*., 1993). It has been reported that super oxide resulting from streptozotocin-intoxicated rats is due to mitochondrial oxidation and increase in xanthine oxidase activity (Szkudelski *et al*., 2001). Thus in order to reduce the effect of streptozotocin, SOD catalyzes the dismutation of the super oxide radical and the CAT removes the product of SOD (hydrogen peroxide) from circulation. The enzymes SOD and CAT are major antioxidant defense systems of the body, which protect the cell membrane and other cellular constituents against oxidative damage by free radical species (ROS) (Umamaheswari and Chatterjee, 2009). Both the extract and the reference drug (glibenclamide) prevented the collapse in antioxidant defense by maintaining the SOD, CAT and MDA towards normal. Earlier reports portray methanolic extract of the stem bark of M. lucida as having radical scavenging activities *in vitro* (Ogunlana *et al*., 2008). Thus, the presence of plant extract or glibenclamide prevented the build-up of oxidative stress and allowed for the recovery from antioxidant collapse. Mechanism to curb the effect of oxidative stress include free radicals scavenging, lipid peroxidation inhibition and many other resulting to disease prevention (Youdim and Joseph, 2001). The ability of *M. lucida* stem bark extract to prevent the alteration of antioxidant status explains their protective role. This may be due to the presence of phenolic content and reducing substances as reported from the phytochemical screening. The phytochemical screening further reveals the presence of saponnins and flavonoids that possess hypoglycemic activity by acting on insulin or by stimulating insulin secretion by the beta cells of the islets of Langerhans (Youdim and Joseph, 2001).

CONCLUSION

The ability of the aqueous stem bark extract of M lucida to decrease the blood glucose of hyperglycemic rats towards normal confirms its antidiabetic activity. The stem bark extract of M lucida also inhibited diabetic complications by preventing an alteration in plasma concentration of creatinine, urea, triglyceride, and cholesterol and also AST and ALT activities. The aqueous stem bark extract of *M. lucida* possesses good antioxidant potentials by inhibiting collapse of antioxidant defense induced by streptozotocin. The bioactive substances earlier identified in this plant material may account for its medicinal and antioxidant properties.

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