

Millettia aboensis Attenuates Diabetes Induced Systemic Oxidative Stress in Experimental Animal Model



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Abstract

Millettia aboensis has both antioxidant and antihyperglycemic potentials. However, no scientific studies have investigated its effect on diabetes induced systemic oxidative stress which is a major driving force of diabetic complications. This study evaluated the effect of *M. aboensis* on diabetes induced systemic oxidative stress in experimental animal model. Diabetes was induced with 70 mg/kg streptozotocin (i.p) following 30 minutes pre-administration of 50 mg/kg nicotinamide (i.p). Diabetic rats were treated orally with 200, 300 and 400 mg/kg of the extract and fractions for 21 days. Fasting blood glucose was determined every 7 days while blood samples were collected on the 22nd day for the determination of antioxidant enzymes and total antioxidant status. The extract showed significant ($p < 0.05$) reduction in blood glucose at 400 mg/kg from the 14th day of treatment while the ethyl acetate fraction at 300 mg/kg showed significant ($p < 0.05$) effect from the 7th day of treatment. Treatment with the extract, ethyl acetate and butanol fraction at all doses showed significant ($p < 0.05$) increase in catalase enzyme activity compared with the diabetes induced control group. Ethyl acetate fraction at all doses also showed significant ($p < 0.05$) increase in superoxide dismutase enzyme activity and total antioxidant capacity. This study validated the antihyperglycemic effect of *M. aboensis* as well as established the ability of this plant extract and fractions to attenuate diabetes induced oxidative stress. *M. aboensis* may be of therapeutic importance in the management of both hyperglycemia and oxidative damage associated with diabetes.

Keywords: *M aboensis*; Diabetes, Oxidative stress; Antihyperglycemic effect

Background

Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia. Defects in insulin secretion and/or insulin action are common factors associated with all forms of diabetes [1]. Evidences from numerous scientific experiments have highlighted the correlation between oxidative stress and diabetes [2-4]. *In vitro* studies with pancreatic beta cells have also provided evidence for an increased reactive oxygen species production in diabetes [5-7]. Hyperglycemia contributes not only to increased free radical generation but has also been established to attenuate enzymatic and non-enzymatic antioxidant mechanisms [8,9]. Due to the highly reactive nature of free radicals, they directly oxidize and damage DNA, proteins and lipids [10]. Increased free radical formation in the absence of appropriate compensatory

antioxidant defence mechanism predisposes to oxidative stress. Hyperglycemia-induced oxidative stress is now recognised as the driving force for the development of diabetic complications [11]. Despite efforts to control blood glucose with current methods of treating diabetes, tissue and organ damages has been observed to keep accumulating over time in most diabetic patients [12]. The beneficial effect of targeting oxidative stress together with hyperglycemia has been reported in animal models of diabetes as well as in diabetic patients [2,3].

In our previous studies, we reported that *M. aboensis* ameliorated site (liver) specific oxidative stress induced by carbon tetrachloride (CCL₄) [13]. The active principles in the extract were also shown to interact synergistically in scavenging

free radicals [13]. On the other hand, the ability of the leaf extract of *M. aboensis* to reduce hyperglycemia in alloxan model of diabetes has been reported [14]. Its pancreatic protective effect has also been associated with its antidiabetic activity [14]. Given that *M. aboensis* has both antioxidant and antihyperglycemic potentials, it is expected that the combination of these effects may be beneficial in reducing complications associated with diabetes. However, no scientific studies have investigated the effect of this plant on diabetes induced systemic oxidative stress which is a major driving force of diabetic complications. To fill the gap in knowledge, this study evaluated the effect of *M. aboensis* on diabetes induced systemic oxidative stress in experimental animal model.

Materials and Methods

Plant material

The leaves of *M. aboensis* were collected from Nsukka, Enugu State, Nigeria and were authenticated by a taxonomist - Mr. Alfred Ozioko of Bioresource Development and Conservation Project, Nsukka, Enugu State, Nigeria. A voucher specimen has been deposited at the herbarium of the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, with the herbarium number PCG/474/A/021. The leaves were air dried and pulverized into coarse powder.

Animals

Wistar Albino rats (200 -250) were used for this study. The animals were obtained from the Animal House of the Department of Pharmacology/Toxicology, Nnamdi Azikiwe University, Awka. All animal experiments were conducted in compliance with NIH guide for care and use of laboratory animals.

Extraction and fractionation

The pulverized leaves were cold macerated in aqueous ethanol (70%) for 48 h and the resulting solution was filtered and concentration in *vacuo* using rotary evaporator at 50°C. Two-third of the ethanol extract was subjected to liquid-liquid partition successively with 2.5 L of n-hexane, ethyl acetate, and then butanol to give different fractions soluble in these solvents. The fractions were concentrated using rotary evaporator at 50°C and stored at 4°C.

Diabetes induced systemic oxidative stress

Albino rats (n = 100) were fasted overnight for 12h and fasting blood glucose levels taken from tail vein using Accu-check Glucometer (Roche, Germany) before the induction of diabetes with 70 mg/kg STZ (i.p) following 30 minis pre-administration with 50 mg/kg NAD (i.p). After 72 h, animals with fasting blood glucose levels > 160 mg/dl were confirmed diabetic and selected for the study. The animals were grouped into fifteen groups of six animals each. Groups 1 - 3 received the extract, groups 4 - 6 n-hexane fraction, groups 7 - 9 ethyl acetate fraction and groups

10 - 12 butanol fraction at the oral doses of 200, 300 and 400 mg/kg respectively. The control groups (13 and 14) received 5 mg/kg glibenclamide and 5 mL/kg 5% Tween 20 respectively. Diabetes was not induced in the 15th group but received 5 mL/kg 5% Tween 20. Treatment with the extract and fractions continued for 21 days with fasting blood glucose determined every 7 days. On the 22nd day, blood samples were collected through retro-orbital plexus into a plain tube. The sera obtained were used for the determination of antioxidant enzymes and total antioxidant status.

Determination of antioxidant enzyme activity

Superoxide dismutase activity was determined by its ability to inhibit the auto-oxidation of epinephrine determined by the increase in absorbance at 480 nm as described by Sun & Zigma [15] while catalase activity monitored by the disappearance of H₂O₂ determined according to the method of Beers and Sizer as described by Usoh et al. [16].

Determination of serum total antioxidant capacity

Total antioxidant capacity of the serum was determined using the method of Kampa et al. [17] based on the ability of antioxidants to inhibit the oxidation of 2,2-Azino-di-3-ethylbenzthiazoline sulphonate (ABTS) by metmyoglobin. Total antioxidant capacity was quantified as millimolar Trolox equivalents.

Statistical analysis

Results were expressed as Mean + SEM. Statistical analysis were done using one way ANOVA followed by posthoc multiple comparism using Turkey's test. Differences between means were considered significant at P < 0.05.

Results

Effect of treatment on STZ-NAD induced hyperglycemia

Hyperglycemia was observed in all groups 72 h post induction with STZ-NAD. This hyperglycemic state was maintained throughout the 21 days the study lasted (Figure 1). The extract at 400 mg/kg on the 14th day after diabetes induction produced significant (p<0.05) reduction of blood glucose while significant (p < 0.05) reduction was observed at all doses on the 21st day. The ethyl acetate fraction at 300 mg/kg showed significant (p<0.05) reduction in blood glucose from the 7th day when compared to vehicle control group (5% Tween 20). At 300 and 400 mg/kg, the ethyl acetate fraction produced significant (p <0.05) reduction in blood glucose compared with 72 h post-induction and control group (5% tween 20) just like butanol fraction at 400 mg/kg.

Effect of treatment on oxidative stress

Induction of diabetes produced significant (p<0.05) reduction in antioxidant enzymes activities as well as total antioxidant capacity compared to un-induced control group. The suppressed antioxidant enzymes and total antioxidant capacity were however

elevated after treatment with the extract and fractions of *M. aboensis* (Table 1). Treatment with the extract, ethyl acetate and butanol fraction at all doses showed significant ($p < 0.05$) increase in catalase enzyme activity compared with the diabetes induced control group. Ethyl acetate fraction at all doses also showed

significant ($p < 0.05$) increase in superoxide dismutase enzyme activity and total antioxidant capacity while the extract and butanol fraction only showed significant ($p < 0.05$) effect at 300 and 400 mg/kg compared with the diabetes induced control group.

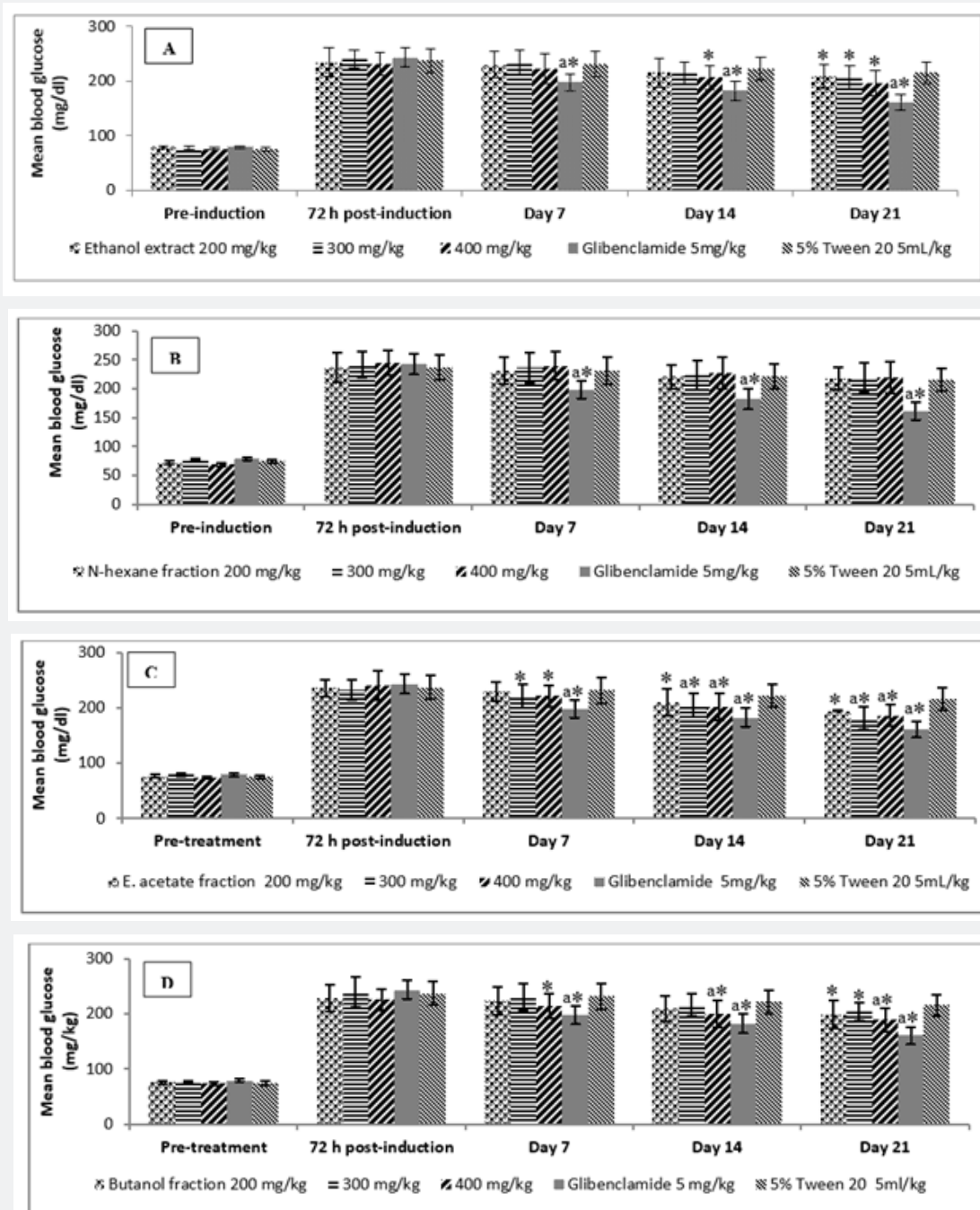


Figure 1: Effect of the extract and fractions on STZ-NAD induced hyperglycemia N = 6, a $p < 0.05$ compared with 72 h post-induction, * $p < 0.05$ compared with 5% Tween 20. A = Ethanol extract, B = N-hexane fraction, C = Ethyl acetate fraction, D = Butanol fraction.

Table 1: Effect of extract and fractions on serum antioxidant enzymes and total antioxidant capacity.

Treatment	Dose (mg/kg)	CAT (U/mL)	SOD (U/mL)	TAC (mM) TE
Ethanol Ext	200	6.1 + 0.1*	3.0 + 0.5	0.55 + 0.04
	300	6.5 + 0.4*	3.5 + 0.3*	0.72 + 0.05*
	400	6.7 + 0.8*	3.6 + 0.5*	0.79 + 0.03*
N-Hexane F.	200	3.5 + 0.2	2.6 + 0.8	0.45 + 0.04
	300	3.9 + 0.5	2.6 + 0.5	0.46 + 0.02
	400	4.1 + 0.9	2.9 + 0.9	0.51 + 0.06
Ethyl acetate F.	200	6.5 + 0.3*	3.5 + 0.6*	0.91 + 0.02*
	300	6.8 + 0.2*	3.8 + 0.2*	0.96 + 0.03*
	400	7.2 + 0.6*	3.8 + 0.4*	1.08 + 0.01*
Butanol F.	200	6.5 + 0.4*	3.2 + 0.7	0.56 + 0.05
	300	6.6 + 0.1*	3.5 + 0.3*	0.77 + 0.02*
	400	6.8 + 0.5*	3.7 + 0.4*	0.81 + 0.04*
Glibenclamide	5	6.9 + 0.6*	3.9 + 0.7*	1.21 + 0.04*
5% tween 20	5 ml/kg	3.6 + 0.5	2.7 + 0.5	0.42 + 0.03
5% Tween 20 (uninduced)	5 ml/kg	12.4 + 0.7*	7.5 + 0.4*	3.96 + 0.10*

n = 6, *p < 0.05 compared with control (5% tween 20), Ext: Extract, F: Fraction, CAT: Catalase, SOD: Superoxide Dismutase, TAC: Total Antioxidant Capacity, TE: Trolox Equivalent

Discussion

Several studies have demonstrated that oxidative stress plays an important role in the pathogenesis of diabetes complications ranging from stroke, neuropathy, retinopathy, nephropathy to cardiomyopathy [11]. In these pathological conditions, hyperglycemia has been seen as causal link between diabetes and increased oxidative stress [9]. Many biochemical pathways strictly associated with hyperglycemia, such as glucose autooxidation, polyol pathway, prostanoid synthesis and protein glycation increases ROS production [18]. Thus, maintaining adequate glucose level in diabetic condition is necessary for combating oxidative stress. The elevation of blood glucose above 160 mg/dl post administration of STZ-NAD was an indication of hyperglycemia associated with the combination of these agents. STZ is known to cause pancreatic β-cell damage whereas NAD partially protects insulin secreting cells against STZ complete damage [19]. STZ-NAD diabetes induction therefore mimics type II diabetes mellitus. The reduction in blood glucose by *Millettia aboensis* may be associated with its phytochemicals. In our previous study, two procyanidins- catechin and epicatechin were isolated from the ethyl acetate fraction of *M. aboensis* [13] which happens to also be the most active antidiabetic fraction in this study. Procyanidins have shown antidiabetic activity in different glucose homeostasis disruption experimental models [20]. Their insulin mimetic effect on the liver and peripheral tissues has been documented as well as improvement of insulin expression in pancreatic β-cell [21-22]. Other mechanism of antidiabetic activity recorded for this class of phenolic compounds include but not limited to improvement of insulin expression in pancreatic

β-cell, decreased β-cell apoptosis, increased glucose uptake and utilization in muscle and liver [20]. Decreased gluconeogenesis and glycogenolysis in the liver have also been reported with extracts rich in procyanidins [23]. The presence of this phytochemical in *M. aboensis* may be responsible for its reduction of blood glucose and may account for the differential activity of the fractions of the extract. Reduction in serum superoxide dismutase and catalase antioxidant enzyme activities as well as serum total antioxidant capacity were indications of compromised systemic oxidative stress following STZ-NAD induced diabetes. These findings were in agreement with other studies that have reported reduction in antioxidant enzymes as a major contributor to oxidative stress in diabetes [9,11]. Improvement in complications of diabetes has been observed following treatment with antioxidants providing evidence that these complications are associated with antioxidant deficiency [24]. Similarly, the therapeutic potentials of improved antioxidant enzyme in diabetic conditions was also demonstrated in studies that showed that over expression of antioxidant enzymes in STZ-treated transgenic mice attenuated the onset of diabetic complications [4]. The improvement in total antioxidant capacity and increased antioxidant enzymes following treatment with extract and fractions of *M. aboensis* is an indication of their ability to attenuate oxidative stress associated with STZ-NAD induced diabetes. This improved oxidative stress is expected to contribute to their beneficial effect in the management of hyperglycaemia associated with diabetes and other diabetic pathological complications mediated through oxidative stress.

Phenolic compounds particularly catechins have been found to increase plasma total antioxidant capacity, attenuated stress-

sensitive signalling pathways and prooxidant enzymes and as well a known inducer of antioxidant enzymes like catalase and superoxidase dismutase [25]. Similarly, there are increasing evidences that as antioxidants, polyphenols protect cell constituents against oxidative damage and therefore through such means limit the risk of disease conditions associated with oxidative stress [26]. Pandey & Rizvi [27] also found that consumption of antioxidants has been associated with reduced levels of systemic oxidative damages. We have previously reported that this plant exhibited site specific oxidative stress on CCL₄ induced liver oxidative stress [13]. The isolated procyanidines were also reported to act in synergy. These same compounds may have also mediated the attenuation of systemic oxidative stress resulting from STZ-NAD induced diabetes.

Conclusion

This study validated the antihyperglycemic effect of *M. aboensis* as well as established the ability of this plant extract and fractions to attenuate diabetes induced oxidative stress. Since oxidative stress mediates most end organ damage and complications of diabetes, *M. aboensis* may be of therapeutic importance in the management of both hyperglycemia and oxidative damage associated with diabetes.

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