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Anti-diabetic and Antioxidant Effects of Methanol Extract of *Dialum guineense* on Alloxan–induced Albino Rats

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ABSTRACT

The effects of methanol extract of *Dialium guineense* (Dg) on antidiabetic and antioxidant properties of alloxan–induced albino rats was evaluated. Twenty-five wistar albino rats were divided into five groups of five rats each. Diabetes was induced in the rats intraperitoneally with 100 mg/kg body weight (bw) of alloxan monohydrate. Group A served as the normal control (received feed and water only) and group B received only alloxan and served as untreated diabetic rats. Group C received 5 mg/kg bw of glibenclamide (standard drug) while groups D and E received 500 mg/kg bw and 1000 mg/kg bw of Dg extract respectively for 14 days. Blood samples were collected by cardiac puncture and activities of superoxide dismutase, catalase and glutathione peroxidase (GPX) and concentrations of reduced glutathione (GSH), malondialdehyde (MDA), fasting blood sugar (FBS), pH, vitamins E and C were estimated using standard biochemical methods. Fasting blood sugar and malondialdehyde concentrations decreased significantly ($p<0.05$) in the groups treated with 500 mg/kg bw and 1000 mg/kg bw of methanol extract of *Dialum guineense* when compared with untreated diabetic rats. Reduced glutathione (GSH) level, glutathione peroxidase (GPX) and catalase activities increased significantly ($p<0.05$) in both groups treated with methanol extract of *Dialum guineense* when compared with untreated diabetic rats whereas superoxide dismutase activity, pH, vitamin C and E levels shows no significant ($p>0.05$) difference in both groups treated with methanol extract of Dg when compared with untreated diabetic rats. These findings suggest that methanolic extract of *Dialum guineense* leaves may have antioxidant properties and may be used in the management of diabetes whose pathogenesis and progression are known to be influenced by oxidant species.

KEYWORDS: Alloxan, Antioxidants, Diabetes, *Dialum guineense*, Oxidative stress

INTRODUCTION

Most plants possess some active therapeutic agents against diseases due to their biodiversity and presence of wide varieties of bioactive phytochemicals and secondary metabolites [1]. Most disease management are done with medicinal plants [2]. Several evaluations of chemical and biological activities of plants have resulted to compounds with properties that is used in the development of modern synthetic drugs for management of several diseases including diabetes [3].

Dialium guineense belongs to the family of Fabaceae. It is a small tree or shrub 10-20 m high with dense crown and hanging leaves. The bark is smooth, greyish, and slash-reddish sometimes exuding a red gum and Stems are pubescent and brown. The leaves are alternate usually with 5-7 opposite or sub alternate leaflets. The flowers are green about 10cm across and 5-6 mm in diameter. The fruits are lenticular or flattened globose, about 2-2.5cm in diameter containing 1or 2 seeds embedded in a reddish acidulous and sweetly edible pulp [4].

Dialum guineense has other common names such as black velvet (English), Icheku (Igbo, Eastern Nigeria), Awin (Yoruba, Western Nigeria), Tamarinier noir (French) [5]. Different parts of the tree have been used in traditional medicine for treatment of various diseases such as cancer, headache and pains (bark), fever, prenatal pains and oedema (leaves), diarrhoea (fruits)[4]. Ezeja*et al*., [5] also reported that *Dialum guineense* demonstrated a significant analgesic activity

Diabetes mellitus is a non-communicable metabolic disorder characterised by hyperglycemia [6-8] and alterations in carbohydrate, lipid and protein metabolism, associated with absolute or relative deficiencies in insulin secretion and/or insulin action [9]. It tends to damage cell membrane which results in elevated production of reactive oxygen species (ROS) [10] and the production of these ROS tend to play a critical role in the pathogenesis of diabetes mellitus [10]. Diabetes is mainly caused by hyperglycemia and also increases production of ROS and affects antioxidant enzymes and reactions [11-13]. It has also been described as a metabolic disorder of multiple etiologies characterized by chronic hyperglycemia with disturbances of carbohydrates, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both [14-16] and the major goals in the treatment of diabetes has been to keep both short term and long term glucose levels within acceptable limits, thereby reducing the risk of

long term complications [17]. It can be induced by alloxan and it does this by destroying the beta cells of the islets in the pancreas thereby leading to decrease in synthesis and release of insulin [18].

Medicinal plants have been used for treatment of diabetes but only a few have been analysed scientifically [19] and also there is high level of treatment failures and unpleasant side effects associated with oral anti-diabetic drugs thereby resulting to an urgent need for alternative treatments [20].

Despite all the researches done so far with this plant, there is scanty information on the antidiabetic and antioxidant activity of the *Dialum guineense* leaves therefore the present study was undertaken to investigate the antioxidant and antidiabetic effects of *Dialum guineense* with the aim of establishing the pharmacological basis for its traditional use to treat diabetes and some underlying complications.

MATERIALS AND METHODS

Collection and Identification of Materials

Dialum guineense leaves were obtained from Nsukka in Enugu State. They were identified by the Herbaruim unit of the Department of Forestry and Environmental Management, Michael Okpara University of Agriculture, Umudike, Umuahia, Abia State.

Preparation of Extract

All the leaves were washed in running tap water, air dried and pulverized. The powdered material was directly macerated in methanol for 72 hours with intermittent shaking to facilitate extraction. The extract was filtered using Whatman filter paper and was evaporated to dryness using water bath at temperature of 40^{o}c. The residue obtained was weighed and dissolved in distilled water to use daily for the experiment.

Experimental Design

Twenty five (25) female wistar albino rats weighing between (100-130 g) were used for this study. The animals were obtained from the Animal Breeding Unit of the College of Veterinary medicine, University of Nigeria, Nsukka. They were kept in well ventilated stainless steel cages and left under laboratory conditions for one week for acclimatization. The animals were divided into five groups (A-E) of five rats each. They were fed with commercial rat feed and clean tap water *ad libitum* throughout the period of experiment. Group A was the control administered

only water and feed, group B was treated with alloxan only, group C was treated with alloxan and standard drug (glibenclamide), group D was treated with alloxan and 500 mg/kg bw of *Dialium guineense* leaves extract and group E was treated with alloxan and 1000 mg/mg bw of *Dialum guineense* leaves extract for 14 days.

Preparation of Drugs

One caplet (50 mg) of the glibenclamide was dissolved in 20 ml of distilled water and was administered orally to the animals based on body weight.

Induction of Diabetes

Experimental diabetes was induced by injecting freshly prepared alloxan monohydrate at 100 mg dissolved in 10 ml of normal saline in a single dose corresponding to l00 mg/kg bw of alloxan. It was administered intraperitoneally according to body weight within few minutes of preparation after base line test has been determined. After 3 days, blood was collected to chemically establish the diabetic condition by making an incision at the tail end using a sharp scissors and a reagent strip. Fasting blood glucose level was determined using a potable glucometer and the blood glucose level of the rats was greater than 185mg/dl after the three days of post induction.

Blood Samples

At the end of the treatment period, rats in all the groups were dissected and blood samples were collected by cardiac puncture in EDTA free bottles for Biochemical assay.

BIOCHEMICAL ANALYSIS

Superoxide Dismutase Determination

The activity of superoxide dismutase was determined using the method of Xin *et al*. [21].

Catalase Determination

The activity of superoxide dismutase was determined using the method of Shina [22]

Glutathione Peroxidise Determination

The activity of glutathione peroxidase was determined using the method of Paglia and Valentine [23].

Reduced Glutathione Determination

Glutathione concentration was determined according to the method of Ellman [24].

Malondialdehyde Determination

Malondialdehyde level was estimated using the method of Wallin *et al*. [25]

Vitamin E Determination

Vitamin E level was determined using the method of Rosemberg [26]

Vitamin C Determination

Vitamin C level was determined using the method of Omaye [27]

pH Determination

pH level was determined by the method of Ball [28]

Statistical Analysis

Descriptive statistics were carried out on the data generated. Results were expressed as the mean ± S.D. One way analysis of variance (ANOVA) was used to separate means with post-hoc multiple comparison (option - LSD). Probability value is less than 0.05 ($p \leq 0.05$). Data analysis was done using SPSS (Statistical package for social scientists).

RESULTS AND DISCUSSION

The result of the present study showed that there was a significant ($p<0.05$) decrease in activities of catalase and GPX and the level of GSH in an untreated diabetic group when compared with the normal control whereas malondialdehyde (MDA), creatinine and fasting blood sugar (FBS) levels increased significantly ($p<0.05$) in an untreated diabetic rats when compared with the normal control. However a significant ($p<0.05$) increase was observed in GPX and catalase activity and GSH level in the group treated with different concentrations (500 mg/kg and 1000 mg/kg bw) of Dg extract when compared with untreated diabetic rats whereas MDA and FBS levels decreased significantly ($p<0.05$) in the group treated with different concentrations (500 mg/kg and 1000 mg/kg bw) of Dg extract when compared with untreated diabetic rats. Superoxide dismutase (SOD) activity and pH, vitamin E and C levels showed no significant difference ($p>0.05$) in group treated with different concentrations of Dg extract when compared with untreated diabetic group but a marked increase was observed.

Table 1: Effect of Methanolic Extract of *Dialium guineense* on Fasting Blood Sugar Level on Alloxan-induced Albino Rats.

GROUP	TREATMENT	FBS
A	Normal control	98.00±06.25[b]
B	Alloxan only	421.80±36.02[a]
C	Glibenclamide	121.50±15.44[b]
D	500mg/kg bw DG	195.00±85.36[b]
E	1000mg/kg bw DG	108.70±09.39[b]

Values are expressed as means ±SD (n=5). Values with superscript letter (b) in the same row are significantly different ($p<0.05$) when comparing group B with other groups.

Table 2: Effect of Methanolic Extract of *Dialium guineense* on Non-Enzymatic Antioxidants (GSH, VIT. C, VIT. E) and MDA Concentrations on Alloxan-induced Albino Rats.

GROUP	TREATMENT	GSH	VIT. C	VIT. E	MDA
A	Normal control	5.35±0.06[b]	1.54±0.11[a]	1.84±0.03[a]	5.13±0.26[b]
B	Alloxan only	3.90±0.09[a]	1.58±0.10a	1.84±0.19[a]	9.88±0.18[a]
C	Glibenclamide	4.78±0.04[b]	1.68±0.08[a]	1.78±0.10[a]	5.30±0.13[b]
D	500 mg/kg bw DG	5.56±0.05[b]	1.65±0.05[a]	1.91±0.09[a]	4.00±0.13[b]
E	1000mg/kgbw DG	5.34±0.10[b]	1.67±0.08[a]	2.00±0.14[a]	3.22±0.11[b]

Values are expressed as means ±SD (n=5). Values with superscript letter (b) in the same row are significantly different ($p<0.05$) when comparing group B with other groups

Table 3: Effect of Methanolic Extract of *Dialium guineense* on Antioxidant Enzymes (SOD, GPX, Catalase) Activities on Alloxan-induced Albino Rats

GROUP	TREATMENT	SOD	GPX	CAT
A	Normal control	1.09±0.02[a]	24.27±1.12[b]	2.85±0.05[a]
B	Alloxan only	1.10±0.00[a]	15.60±0.51[a]	2.45±0.11[a]
C	Glibenclamide	1.12±0.12[a]	27.86±1.00[b]	3.58±0.58[b]
D	500mg/kg bw DG	1.15±0.14[a]	31.19±1.38[b]	5.27±0.70[b]
E	1000mg/kg bw DG	1.16±0.15[a]	51.63±0.32[b]	6.23±0.80[b]

Values are expressed as means ±SD (n=5). Values with superscript letter (b) in the same row are significantly different ($p<0.05$) when comparing group B with other groups.

Table 4: Effect of Methanolic Extract of *Dialium guineense* on Non-Enzymatic Antioxidant (GSH, VIT. C, VIT. E) and MDA Concentrations on Alloxan-induced Albino Rats

GROUP	TREATMENT	GSH	VIT. C	VIT. E	MDA
A	Normal control	5.35±0.06^{b}	1.54±0.11^{a}	**1.84±0.03^{a}**	5.13±0.26^{b}
B	Alloxan only	3.90±0.09^{a}	1.58±0.10a	1.84±0.19^{a}	9.88±0.18^{a}
C	Glibenclamide	4.78±0.04^{b}	1.68±0.08^{a}	1.78±0.10^{a}	5.30±0.13^{b}
D	500mg/kg bw DG	5.56±0.05^{b}	1.65±0.05^{a}	1.91±0.09^{a}	4.00±0.13^{b}
E	1000mg/kgbw DG	5.34±0.10^{b}	1.67±0.08^{a}	2.00±0.14^{a}	3.22±0.11^{b}

Values are expressed as means ±SD (n=5). Values with superscript letter (b) in the same row are significantly different ($p<0.05$) when comparing group B with other groups.

Table 5: Effect of Methanolic Extract of *Dialium guineense* on pH Level on Alloxan-induced Albino Rats

GROUP	TREATMENT	pH
A	Normal control	7.70±0.15^{a}
B	Alloxan only	7.60±0.14^{a}
C	Glibenclamide	7.60±0.14^{a}
D	500mg/kg bw DG	7.80±0.16^{a}
E	1000mg/kg bw DG	7.70±0.15^{a}

Values are expressed as means ±SD (n=5). Values with superscript letter (b) in the same row are significantly different ($p\leq0.05$) when comparing group B with other groups

A significant increase ($p<0.05$) was observed in the level of fasting blood sugar in group administered alloxan only when compared with the normal control, the increase in blood glucose levels may be due to the severe damage to the pancreas as a result of the interference in the metabolic pathway of carbohydrate [29], but treatment with different concentrations of the extract resulted to a significant decrease ($p<0.05$) in fasting blood sugar level when compared with untreated diabetic rats, this reduction of blood glucose level may be due to inhibition of glucose absorption which increases sensitivity of receptors to insulin and stimulation of peripheral glucose uptake [30] and may also be due to the regeneration of beta cells of the pancreas by the presence of antioxidants present in *Dialum guineense* leaves, this result also agrees with the work of [31] who reported that the presence of antioxidants present in *Justicia carnea* may be the reason for the reduction of fasting blood glucose after treatment.

Accumulation of reactive oxygen species and its changes in the cellular system are mainly caused by oxidative stress [32]. Antioxidant enzymes and compounds in cell protect major macro molecules from oxidative damage caused by reactive oxygen species [33], also hyperglycemia can increase oxidative stress and change the redox potential of glutathione [34]. Decreased level of GSH observed in untreated diabetic rats when compared with normal control may increase their susceptibility to oxidative injury [35]. Reduction of oxidised form of glutathione requires NADPH, as a cofactor and enzyme glutathione reductase. The reduced availability of NADPH, which could be either due to reduced synthesis or increased metabolization of NADPH through some other pathway, could be also responsible for low levels of reduced glutathione in alloxan diabetic rats as compared to control rats [36]. Other researchers have also reported the decreased level of liver and kidney GSH in alloxan induced diabetic rats [37] but upon treatment with different concentrations of *Dialum guineense*, the GSH increased significantly ($p<0.05$) relative to untreated diabetic rats this may be due to less production of ROS [35] as a result of the antioxidants and some photochemicals present in the plant.

Lipid peroxidation (LPO) is the process whereby oxygen interacts with polyunsaturated fatty acids and gross alteration of structural, organizational and enzyme function usually happens when this process occurs in biological membrane. Furthermore, lipid peroxide mediated damage has been observed in the development of type 1 and type 2 diabetes mellitus [38]. It is also a measure of oxidative stress [39] and it is often measured by the production of MDA; a marker of lipid peroxidation. A significant ($p<0.05$) increase was observed in MDA level of untreated diabetic rats relative to the normal control, this may be suggestive of increased oxidative stress and depletion of reduced glutathione leading to diabetes. Irshad *et al*. [34] reported that hyperglycaemia can increase oxidative stress, also the reduction of two electrons from alloxan gives dialuric acid, which undergoes oxidation and leads to generation of O_2 •–, H_2O_2 and OH•. Dialuric acid has been observed to stimulate lipid peroxidation *in vitro* [40]. However treatment of the rats with different concentrations of

Dialum guineense extract successfully reversed the situation by decreasing the level of MDA relative to the untreated diabetic rats, this may indicates that *Dialum guineense* extract may scavenge or inhibit the free radical formation, this also corroborate with the work of Raghavan and Kumari [35].

Superoxide dismutase catalyses the dismutation of superoxide radicals and generate hydrogen peroxide which is also decompose by catalase producing molecular oxygen and water which are toxic [41].Glutathione peroxidase (GPX) catalyses the reduction of lipid hydroperoxide to their corresponding alcohols and also reduces free hydrogen peroxide to water. In this present study, catalase and glutathione peroxidise (GPX) activities decreased significantly in the untreated diabetic rats when compared with normal control. This significant decrease may be due to high levels of reactive oxygen species generated as a result of diabetes caused by alloxan. Raghavan and Krishnakumar [35] reported that the decrease in the activities of these (GPx, GST, GR) enzymes result in the involvement of deleterious oxidative changes and also insufficient availability of GSH. However significant increase in the activities of these antioxidant enzymes (CAT and GPX) in the group administered with methanolic extracts of Dg is a suggestive of protection of the cells from oxidative damage caused by these reactive species. Our findings corroborate with work of Ukpabi-Ugo *et al.* [33] who reported that a significant rise in the activity of antioxidant enzymes protect the cell from oxidative damage caused by reactive oxygen species.

Superoxide dismutase (SOD) activities and non-enzymatic antioxidants (Vitamin C and D) levels shows no significant difference ($p>0.05$) in the untreated diabetic rats when compared with the normal control and also no significant difference ($p>0.05$) was observed in the pH level of the rats which shows that the extract and alloxan does not have any effect on the pH level of the albino rats.

CONCLUSION

These findings suggest that methanolic extract of *Dialum guineense* has antioxidant properties and can be used in the management of diabetes whose pathogenesis and progression are known to be influenced by oxidant species

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