ANTIOXIDATIVE AND ANTI-HYPERGLYCAEMIC EFFECT OF *CARALLUMA DARZIELII* IN ALLOXAN INDUCED DIABETIC RATS

[*1]Ahmad, M. B., [2]Gwarzo, M. Y., [3]Hadiza, L. A., [4]Adamu, A. I. and [5] Halliru, A. H.
Department of Medical Laboratory Science, BUK, Kano, Nigeria
*Corresponding address: mohammadahmadbello@yahoo.com +234806519640

Abstract
Introduction: Diabetes mellitus disease in all its form imposes high human, social and economic costs on countries at all income levels. Plants were used widely as medicinal sources in most developing countries as a common remedy for the maintenance of good health.
Aim: The study investigated the effect *of Caralluma Darzielii* plant extract on hyperglycaemia and lipid peroxidation in an alloxan induced diabetic rats.
Methods: The rats were fed on commercial diet and grouped in to 4. DT as group1, diabetic treated with plant extract, PC as group 2 diabetic positive controls on normal diet, NC as group 3 non diabetic on normal diet and CT as group 4 diabetic treated with chlorpropamide. A 180mg per Kg body weight of alloxan was injected intra-peritoneally which successfully induced the diabetic state. A one week assessments using fasting blood glucose was embarked to ascertain the sustenance and severity of diabetes. The plant extract was then administered to the diabetic induced rats at 100mg/Kg body for four weeks after which a serum glucose level was assessed at weakly intervals. Malondialdehyde was also measured for anti-oxidative effect.
Results: The results indicated that the extracts possess significant hypoglycemic effect with 4.9 ±0.2 vs. 16.3±0.2 mmol/l on the DT group versus PC group respectively ($p<0.05$). A raised malondialdehyde was also observed among the PC with 23.2±3.5 as against 12.5±0.5 mmol/L DT group, ($p<0.05$).
Conclusion: This indicated that *Caralluma Darzielii* plant extract has a potential hypoglycemic and antioxidative property. Further evaluation of active ingredients and chronic complication are therefore recommended
Key words: *Caralluma Darzielii,* Antioxidant, Hyperglycaemia, Lipid peroxidation, Alloxan,

Introduction

Diabetes mellitus results from variable interaction of hereditary and environmental factors (Baynes, 1991). It is a chronic metabolic syndrome characterized by abnormal insulin secretion or its receptor and is a major health problem worldwide (Gwarzo *et al.,* 2014). This affects carbohydrates, proteins and fats metabolism with associated injury to liver, kidney and pancreatic cells (Tanko *et al.,* 2012). The disease in all its form imposes high human, social and economic costs on countries at all income levels. Recently, an estimate indicated that about 382 million people have diabetes worldwide and is projected to rise beyond 592 million in less than 25 years with a vast number progressing towards complications unaware (IDF, 2013). About 175 million of other cases were currently undiagnosed (IDF, 2013).

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All types of diabetes require close collaboration between those affected and their healthcare providers in order to prevent a range of costly and dangerous complications. This can provoke damage to the eyes, kidneys, feet and heart and if left untreated lead to an early death. Evidence implicates elevated extra- and intra-cellular glucose concentrations in a derived oxygen free radicals or oxidants as mediators of diabetic complications (Bonnefont-Rousselot *et al.,* 2000*;* Antonio, 2003*).* Antioxidants mostly derived from plants phytochemicals may likely overcome the imbalance created to cause oxidative stress and or reduce the rising blood glucose among diabetic patients.

Herbal practices are important in providing ways to new areas of research and biodiversity conservation. Plants were used widely as medicinal sources in most developing countries as a common remedy for the maintenance of good health (Musa *et al.,* 2009; Ranjan *et al.,* 2010). Evaluating plant extracts has led to the discovery of many clinically useful drugs that play important roles in the treatment of human diseases (Kumar *et al.,* 2009) and to avoid toxicity of botanical medicine which has been overlooked as health problem (Marcus and Grollman, 2015).

Caralluma darzielii of family *Asclepiadaceae*, is a succulent herb commonly found in the sahelian region of West Africa. It is perennial, erect and sparsely-branched to 40 cm high, with green stems, quadrangular branches and scattered dark reddish-purple star-like flowers (Umar *et al.,* 2013). The plant is called "Karan Masallaci" by the Hausas and "gubehi" by the Fulanis in Northern Nigeria (Burkill, 1985). It occurs from Senegal east to Somalia and also Saudi-Arabia, Yemen, India, northern Nigeria and Sri-Lanka (Umar *et al.,* 2013). It is used in folk medicine as antispasmodic and analgesic remedy (Marinella *et al.,* 2005). It is planted in garden as an ornamental and as a charm against evil (Burkhills, 1985). The latex from heated stem is used as ear drop or wound to treat infection and applied to teeth against dental caries. The crushed aerial parts together with the leaves of *Ozoroa Insignis Dillies* are taken by children to treat coughs (Abdel-Sattar *et al.,* 2009). In dry rural India the plant is cooked and eaten with spices as a vegetable, it is eaten raw by labourers as an appetite and thirst suppressant and endurance enhancer. It is also used to treat chest and cardiac problems (Gilbert, 1990; Abdel-Sattar *et al.,* 2009). The latex is also applied to bites and stings of venomous animals, including spider, ant, scorpions and snakes (Kumar *et al.,* 2009: Umar *et al.,* 2013). This study was conducted to assess the herbalist claim on the hypoglycaemic effect of plant and its activity as antioxidant.

MATERIALS AND METHODS

Plant material

Fresh whole plant of Caralluma dalzielii was collected around Gwarzo road, Kano, Northern Nigeria and was authenticated by the herbarium section, Department of Biological Sciences, Bayero University, Kano.

Preparation of extract

The fresh plant was air dried under the shed at room temperature and grounded into powder. Three hundred and sixty four grams of the fine powder was cold macerated with 70% ethanol for 5 days. The extract was concentrated to dryness on a water bath at 37°C to a mass extract. The extract was reconstituted in distilled water at appropriate concentrations for the various experiments.

Phytochemical screening

The method described by Trease and Evans (1983) was adopted for the caralluma darzielii extract (CDEx) for phytochemical preliminary screening on carbohydrates, flavonoids, alkaloids, saponins, tannins, anthraquinones and glycosides.

Assessment for LD_{50} toxicity

The method of Lorke (1983) for acute toxicity study of LD50 was adopted and was conducted in two stages. Rats were divided into 3 groups of three rats each in the initial phase and treated at doses of 10, 100 and 1000 mg/kg CDEx intra-peritoneally (IP). They were kept under observation for signs of toxicity or death within 24 hours. In the second stage, mice were divided into 4 groups of one mouse each and treated with CDEx at doses of 140, 225, 370 and 600 mg/kg, respectively. The LD50 was calculated from the results of the second stage as the square root of the product of the lowest lethal dose and the highest non-lethal dose as the geometric mean of the consecutive doses with 0 and 100% survival, respectively (Lorke, 1983).

Experimental animals

Wister rats were obtained from the Pharmacology department animal house of college of health sciences, BUK weighting between 120 to 200 g of both sexes and were used for the experiments. They were kept under normal laboratory conditions of humidity, temperature and light for 7 days prior to the experiment and allowed free access to food and water for acclimatization.

Experimental design

The rats were grouped into 4, with ten rats in each group. Each of the rats in a group was weighed after the grouping. Group DT (diabetic and treated) Alloxan induced diabetic rats and treated with 100 mg/kg body weight (bwt.) of methanolic extract of caralluma darzielii. Group PC (positive control) induced diabetic rats but not treated, Group NC (negative control) non diabetic and untreated rats, Group (CT) Diabetic treated with Chlorpropamide

Induction of Diabetes Mellitus

Diabetes was induced by a single dose of 100mg/kg body weight of alloxan monohydrate in freshly prepared 10 mmol/L sodium citrate, pH 4.5, IP, to rats fasting for at least 10 hours. Blood glucose levels were measured 3 days prior to induction and 7 days after of induction. Development of diabetes mellitus was proven by sustained hyperglycemia (>11.11mmol/L).

Biochemical analysis

Estimation of Plasma Malondialdehyde (MDA)

Plasma malondialdehyde was measured by the method of Ohakawa *et al*. (1999). Lipid peroxidation generates peroxide intermediates which upon cleavage release MDA, a product which reacts with Thiobarbitutic Acid (TBA). The product of the reaction is a coloured complex, which absorbs light at 532nm

Methods

A 0.20 cm^3 plasma was dispensed in a test tube containing 3.0 cm^3 of glacial acetic acid to which 3.0 cm^3 of 1% TBA in 2% NaOH was added. The mixture was placed in boiling water for 15 min. Absorbance of the pink coloured product was read at 532 nm after cooling. Calibration curve was constructed using malondialdehyde tetrabutyl-ammonium salt obtained from Sigma (St Louis USA).

Blood Glucose Level estimation (BG)

Blood samples of the rats were collected by cutting the tail tip of the rats for blood glucose determination before administering the extract. Administration of the extract or Chlorpropamide commenced 7 days after induction for a period of 28 days. Blood glucose level was determined based on Glucose Oxidase Method (7) and results were reported as mmol/L.

Statistical data analysis

The mean and standard error of means were obtained for all data analysis using spss 16 programme and for the analysis of variance (ANOVA) to determine the level of significant. P values less than 0.05 ($p<0.05$) were considered significant.

RESULTS

Table 1: Phytochemical composition of ethanolic extract of *Caralluma dalzielii (CDEx)*

Constituents	Inference
Alkaloids	-
Glycosides	+
Flavonoids	+
Carbohydrates	+
Tannins	+
Saponin	+
Steroids	+
Anthraquinones	+

Table 2. Fasting Blood glucose (mmol/L) levels in Alloxan Induced Diabetic Rats Treated with CDEx

GROUPS	INDUCTION	WEEK1	WEEK 2	WEEK 3	WEEK 4
DT	245.00 ±8.75a	163.80 ± 8.42b	133.20 ± 7.55b	105.50 ± 11.42b	98.38 ± 3.08b
DC	233.32 ± 5.25a	238.14 ± 5.59a	229.13 ± 3.12a	247.20 ± 5.83a	244.23±2.98a
NC	132.80 ± 5.02b	122.90 ± 2.49b	118.60 ± 6.78b	137.24 ± 1.56b	122.20±3.52b
CP (84mg/kg)	231.25 ± 4.60a	170.00 ± 1.29b	131.78 ± 1.54b	132.88 ± 2.33b	109.12±1.22b

DT, diabetic treated; PC, diabetic untreated; NC, non-diabetic untreated Values are mean ± standard error of mean (n=4); CP, Chlorpropamide; a=Statistically different (p<0.05) Compared with NC; b=Statistically different (p<0.05) compared with DC.

Table 3. Fasting Blood Glucose (mmol/L) levels after withdrawal of treatment with CDEx

Group	Week 1	Week 2
DT	105.620 ± 9.10b	101.30 ± 6.44b
DC	247.15 ± 14.20	235.82 ± 15.74
NC	101.37 ± 7.21b	105.89 ± 3.11b
CP	110.13 ± 2.20b	120.23 ± 2.21b

DT, diabetic treated; DC, diabetic untreated; NC, negative control; CP, chlorpropamide; Values are mean ± standard error of mean (n=4); b=Statistically significant (p<0.05) compared with diabetic control (DC).

Table 4. Malonyldialdehyde Concentrations in Alloxan Induced Diabetic Rats and Controls after 28 Days of Treatment

GROUP	MALONDIALDEHYDE(mmol/L)
DT	9.5 ± 0.86b
DC	18.3 ± 1.26
NC	8.5 ± 0.76b
CP	17.2 ± 0.76a

DT, diabetic treated; DC, diabetic untreated; NC, non-diabetic untreated; CP, Chlorpropamide.

Values are mean ± standard error of mean (n=4). a=Statistically significant (p<0.05) compared with diabetic control (NC). b=Statistically significant (p<0.05) compared with non-diabetic control (DC).

Discussion

Diabetic mellitus is a global condition known with complications initiated by free radicals as a result of impaired glucose metabolism (Kumar *et al.,* 2005). Considerable number of people in developing countries depends entirely on herbal intervention for their daily health care needs (Marcus and Grollman 2015). Hence, there is an urge to intensify researches into medicinal plants especially to those that are likely to reduce the burden in serious disorders such as diabetes mellitus. *Caralluma darzielii* is believed by herbalists to be used locally for the treatment of diabetes mellitus. There is a significant reduction in mean serum glucose observed among the extract treated group (5.91±0.17) when compared to the mean serum glucose of the diabetic control group (14.51±0.18) mmol/L ($p<0.05$). The anti-diabetogenic effect of the plant extracts was observed throughout the period of administration. Phytochemical screening revealed the presences of flavonoids, tanins, saponins, glycosides and triterpenes steroids cardolides and carbohydrates in the plant extract that have led to its pharmacological activities similarly reported by Tanko *et al.,* 2012. Flavonoids present in the extract might be the molecule of concern to the anti-hyperglycaemic property as it inhibits glucose-6-phophatase activity in a liver by suppressing gluconeogenesis and glycogenolysis which consequently reduces the hyperglycaemia. This might explain the progressive and sustained reduction effect of the extract on serum glucose among DT group. Plants extracts with similar compositions were seen to have same biochemical effect on hyperglycaemia as reported by Rajendran et al., in 2007. Malondialdehyde as a product of lipid peroxidation (Ohakawa et al., 1999) if present in circulation indicates oxidative stress of which has been attributed to initiate and increase the progressive rate of diabetic complications. The MDA level across the set groups were significantly higher in CP (18.3 ± 1.26) and DC (17.2 ± 0.76) compared to that of DT (9.5 ± 0.86) and NC (8.5 ± 0.76) mmol/L ($p<0.005$) groups statistically. *Caralluma darzielii may* possesses potent co-factors among the minerals required that influences the levels of endogenous antioxidants such as superoxide dismutase, catalase and glutathione to down-regulate the level of thiobarbituric acid-reactive substance and hyperglycemia.

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