Effect of ethanol extract of *Caralluma dalzielii* N.E.Br.(Asclepiadaceae) on blood glucose levels of fructose -induced insulin resistance in laboratory animals

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ABSTRACT

The treatment of type 2 diabetes mellitus has been a great challenge, especially in developing countries. Thus the need for plant based substitutes to overcome this problem is necessary due to the side effects of synthetic anti-diabetic drugs. Three doses of the extract Caralluma dalzielii 50, 100 and 200mg/kg B.w. were administered orally to fructose induced types 2 diabetes in Wistar rats . The dose of 50mg/kg bodyweight when administered to diabetic rats, produced an appreciable hypoglycemic effect with (-14.13%) glycemic decrease. Also the standard reference drug glibenclimide produced hypoglycemic effect with (-9.78%) glycemic decrease after 3 days of treatment (p<0.05). As regards the other two doses of the extract, the doses 100 and 200 mg/kg bodyweight produced no significant change in the of blood glucose levels after 3,6 and 9 days of administration, while the least dose of 50mg/kg bodyweight produced a significant decrease (p<0.05) after 9 days of extract administration with glycemic decrease of (-16.31%.)In conclusion, the dose of 50 mg/Kg of the extract has shown both significant (p<0.05) hypoglycemic and anti-hyperglycemic effects in Wistar rats.

Keywords: Fructose, Blood glucose, Diabetes mellitus, *Caralluma dalzielii,* Ethanol, Fructose.
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INTRODUCTION

Diabetes mellitus (DM) currently is a major health problem of the world and is a chronic metabolic syndrome resulting from a variable interaction of hereditary and environmental factors and is characterized by abnormal insulin secretion or insulin receptor or post-receptor events, affecting metabolism involving carbohydrates, proteins and fats in addition to damaging liver, kidney and β-cell of pancreas[1]. Diabetes is a disorder that affects the way your body uses food for energy. Normally, the sugar you take in is digested and broken down to a simple sugar, known as glucose. The glucose then circulates in your blood where it waits to enter cells to be used as fuel. Insulin, a hormone produced by the pancreas, helps move the glucose into cells. World health organization (WHO) report approximately 150 million people have diabetes mellitus worldwide, and this number may well double by the year 2025. Many drugs and interventions are available to manage diabetes; these are expensive for developing countries apart from their inherent adverse effects [2].

Epidemiological studies suggest that insulin resistance is not only an independent risk factor that induces type 2 diabetic mellitus, but also the common cause of hypertension, coronary heart disease, and cerebral vessel disease, thus the key to cure and prevent heart and cerebral vessel disease[3]. Diagnosing diabetes mellitus is not difficult to

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do. Symptoms usually include frequent urination, increased thirst, increased food consumption, and weight loss. The standard criterion for a diagnosis of diabetes is an elevated plasma glucose level after an overnight fast on at least two separate occasions. Glucose value above 126 mg/dl (7.0mmol/L) is often used as the diagnostic value. Diabetes mellitus is a heterogeneous disorder. The causes, symptoms, and general medical outcomes are variable. Generally, the disease takes one of two forms, type-1 diabetes or type-2 diabetes [4].

Insulin resistance is a condition in which the body produces insulin but does not use it properly. As the blood glucose level rises after a meal, the pancreas releases insulin to help cells take in and use the glucose. When people are insulin resistant, their muscle, fat, and liver cells do not respond properly to insulin. As a result, their bodies need more insulin to help glucose enter cells. The pancreas tries to keep up with this increased demand for insulin by producing more. Eventually, the pancreas fails to keep up with the body's need for insulin. Insulin resistance increases the chance of developing type-2 diabetes and heart disease [5].

Fructose causes metabolic syndrome because of its unique metabolism that results in intracellular ATP depletion, uric acid generation, endothelial dysfunction, oxidative stress, and lipogenesis [6,7]. Excess fructose consumption has been hypothesized to be a cause of insulin resistance, obesity[8] elevated LDL cholesterol and triglycerides, leading to metabolic syndrome[9]. Fructose consumption has been shown to be correlated with obesity [10, 11] especially central obesity which is thought to be the most dangerous kind of obesity. A study in mice showed that a high fructose intake increases adiposity [12].

Caralluma dalzielii is a succulent perennial, erect sparsely-branched to 40 cm high, with quadrangular branches, of dry Sahel locations in Senegal to north-western Nigeria, and also in the Sahara region. *Caralluma dalzielii* is cactus like in shape. Its stems are smooth, light green and quadrangular with coarsely crenate to undulate margins between the faces. Its individual flowers are deep purple, very smelly (pollinated by flies), and consist of 5 triangular purple petals and 5 sessile stamens fused to the rim of a white 5 lobed stigma [13].

MATERIALS AND METHODS

Plant material
Caralluma dalzielii sample was collected from Samaru-Zaria in the month of June 2011 and was authenticated by A.U. Gallah of the Biological Sciences Department, Ahmadu Bello University, Zaria where a voucher specimen (No. 1897) was deposited.

Extraction
The extract was prepared in the Department of pharmacology and drug administration, Faculty of Pharmaceutical sciences, Ahmadu Bello University, Zaria. About 100g of the dried plant (*Caralluma dalzielii*) was crushed, using a pestle and mortar. It was then macerated with 70% ethanol for 48 hours. It was then filtered, and the filtrate was evaporated at about 37°C to yield a dark brown mass which weight 60 g and kept in dessicator until use.

Animals
Wistar strain albino rats of both sexes weighed between 120 – 150 g, which were bred in the Department of Pharmacology A. B.U Zaria. The animals were housed in standard environmental conditions of temperature, humidity and a 12 h light-dark cycle. The animals were divided into extract treated groups and the control groups. All the animals were fasted for 12 h, but were allowed free access to water, before commencement of the experiments. This research was carried out in Ahmadu Bello University Zaria, Nigeria in accordance with the rules governing the use of laboratory animals as accepted internationally.

Drugs
All chemicals and drugs were obtained commercially and were of analytical grade.

Phytochemical screening
Phytochemical screening of the extracts was performed for the presence of secondary metabolites using the following reagents and chemicals: alkaloids - with Mayer's and Dragendorff's reagents[14,15] flavonoids with the use of Mg and HCl[16,17] tannins with 1% gelatin and 10% NaCl solutions and saponins with ability to produce suds[17].

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Acute toxicity study
The lethal dose (LD_{50}) of the plant extract was determined by method of Lorke[18] using 12 rats. In the first phase, rats were divided into 3 groups of 3 rats each and were treated with the extract at doses of 10, 100 and 1000mg/kg body weight orally. They were observed for 24 hours for signs of toxicity. In the second phase, 4 rats were divided into 4 groups of 1 rat each and were also treated with the extract at doses of 1,600, 2,900 and 5,000mg/kg body weight orally. The median lethal dose was then calculated.

Induction of Diabetes mellitus and Experimental design
Diabetes was induced in rats by feeding the animals with 20% fructose in the rats' drinking water for 6 weeks.

The fructose induced diabetic Wistar rats were randomly assigned into five groups (1-5) of five rats (n=5) each as follows:
- A.   Group 1 received normal saline.
- B.   Group 2 received 1mg/Kg body weight of Glibenclamide.
- C.   Group 3 received 200mg/Kg body weight of *Caralluma dalzielii.*
- D.   Group 4 received 100mg/Kg body weight of *Caralluma dalzielii.*
- E.   Group 5 received 50mg/Kg body weight of *Caralluma dalzielii.*

Determination of blood glucose levels
All blood samples were collected by cutting the tail-tip of the rats. Blood samples for blood glucose determination were collected from the tail at intervals of 0, 3, 6, and 9 days. Determination of the blood glucose level was done by the glucose-oxidase principle[19] using the Accu-check Advantage glucometer. instrument and results were reported as mg/dl [20]

Statistical analysis
All the data are expressed as mean ± SEM. Statistical comparisons were performed by one way analysis of variance (ANOVA) followed by Duncan's multiple range tests [21]. The results were considered statistically significant if the p values were 0.05 or less. The data were analyzed using SigmaStat v2.0 (Jandel Scietific, Palo Aho, CA, USA).

<div align="center">RESULTS</div>

Phytochemical analysis

<div align="center">Table 1:Preliminary phytochemical analysis of ethanolic extract of Caralluma dalzielii</div>

Constituents	Inference
1. Alkaloids	-
2. Cardenolides	+
3. Carbohydrates	+
4. Flavonoides	+
5. glycosides	+
6. Saponins	+
7. Tannins	+
8. Triterpenes	+
9. Steroid	-

Key: positive (+) = present,   negative (-) = absent

The preliminary phytochemical screening of the extract revealed the presence of cardenolides, carbohydrates, flavonoids, glycosides, saponins, tannins and triterpenes. Also absences of alkaloids and steroids as showed in table 1 above

Acute Toxicity Studies.
The acute toxicity studies, phase 1 shows no lethal dose for 10, 100, and 1000mg/kg. While in the phase 2, 1600, 2900 and 5000mg/kg were administered. 1600mg/kg had no lethal dose but 2900 and 5000mg/kg bodyweight both had lethal doses where the rat in each group died. The lowest lethal dose is 2900mg/kg and the highest non-lethal dose is 1600mg/kg, thus the LD50 = 2,154 mg/kg bodyweight

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The signs of toxicity were first noticed after 4-5 hours of extracts administration. There were decreased locomotor activity and sensitivity to touch and pain. Also there was decreased feed intake, tachypnoea and prostration after 8-12 hours of extracts administration. Early deaths were recorded after 12 hours and late deaths 48hours after extract administration.

Table 2 Effect of *Caralluma dalzielii* on blood glucose levels of fructose-induced diabetic Wistar rats

GROUP	Treatments given	Blood glucose levels (mg/dl)			
		Fasting	Day 3	Day 6	Day 9
1	Normal saline	200.6 ± 9.84	188.2 ± 4.98	182.4 ± 2.31	197.4 ± 5.10
2	Glibenclamide 1mg/kg	179.4 ± 2.03	169.8 ± 4.55^a (-9.78%)	181.6 ± 4.73^{ns} (-0.44%)	186.0 ± 4.37^{ns} (-5.78%)
3	*Caralluma dalzielii* 200mg/kg	201.0 ± 3.17	187.4 ± 6.83^{ns} (-0.43%)	195.8 ± 5.58^{ns} (7.35%)	207.6 ± 11.08^{ns} (5.17%)
4	*Caralluma dalzielii* 100mg/kg	198.2 ± 3.26	195.0 ± 1.67^{ns} (3.61%)	193.0 ± 2.86^{ns} (5.81%)	193.4 ± 3.61^{ns} (-2.03%)
5	*Caralluma dalzielii* 50mg/kg	183.0 ± 10.6	161.6 ± 1.79^a (-14.13%)	157.4 ± 7.18^{ns} (-13.71%)	165.2 ± 7.17^a (-16.31%)

Blood glucose levels are given as mean ± standard error of mean (SEM) for five rats in each group. Experimental groups are compared with diabetic control group at $p<0.05$

Key: "a" = significant
         "ns" = not significant
         Parenthesis ( ) = % decrease in blood glucose level

% Glycemic change = $\dfrac{\text{Mean blood glucose (control)} - \text{Mean treated blood glucose(,3,6 and 9 days)}}{\text{Mean blood glucose (control)}}$ X 100

The blood glucose levels where measured at 0, 3, 6 and 9 days. The levels of significant change in the blood glucose levels were compared between the treated groups and the control group where $^aP<0.05$ is considered as significant and ^{ns} considered as non-significant.

Table 2 showed the results of the effects of three doses (50mg/Kg, 100mg/Kg and 200mg/Kg) of the ethanol extract of *Caralluma dalzielii*, glibenclimide and control groups in fructose diabetic Wistar rats. The doses of glibenclimide and 50mg/Kg of the extract show significant decrease ($p<0.05$) at 3 day of treatments in the blood glucose levels when compared to untreated control. After 6 days of treatments there was no any significant change in all the treated groups when compared to control untreated group. However, the doses of 50mg/Kg of the extract showed a significant ($p<0.5$) decrease in the blood glucose levels after 9 days of treatments.

DISCUSSION

Fructose causes metabolic syndrome because of its unique metabolism that results in intracellular ATP depletion, uric acid generation, endothelial dysfunction, oxidative stress, and lipogenesis[6,7].

Type II diabetes is associated with increased plasma insulin concentration (hyperinsulinemia) which occurs as a compensatory response by the pancreatic beta cells for diminished sensitivity of target tissues to the metabolic effects of insulin, a condition referred to as insulin resistance. The decrease in insulin sensitivity impairs carbohydrate utilization and storage, raising blood glucose and stimulating a compensatory increase in insulin secretion.

Development of insulin resistance and impaired glucose metabolism is usually a gradual process, beginning with excess weight gain and obesity. Excess fructose consumption has been hypothesized to be a cause of insulin resistance obesity [8] elevated LDL cholesterol and triglycerides, leading to metabolic syndrome [9].

The median lethal dose of the extract was 2.154mg/kg. Phytochemical screening revealed the presences of flavonoids, tanins, saponins, glycosides and triterpenes steroids cardolides and carbohydrates.

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After 1 day the mean 200.6±9.84 for the control group when compared with the treated groups there was no significant decrease. At 3 days of treatments the mean control group 188.2± 4.98 when compared with the standard drug (Glibenclimide) at the dose of 1mg/kg and the extract at the dose of 50mg/kg bodyweight there was significant decrease in the blood glucose levels (p<0.005) when compared to control with percentage of glycemic decrease of -9.78% and -14.13% respectively. After 6 days treatments the mean control 182.4±2.31 was compared with the treated groups there was no significant decrease in all the treated groups. While after 9 days of treatment 197.4± 5.10 for the control group there was significant decrease in the only group treated with the extract at the dose of 50mg/kg bodyweight with percentage glycemic decrease of -16.31% The extract is therefore said to have some degree of hypoglycemic activity that is dose dependent, having more effect at lower dose. This finding indicates that the extract could increase the rate of protein synthesis. Enhanced protein synthesis seems to be associated with the administration of the extract and may probably be attributed to improvement in glycemic control or insulin secretion. The hypoglycemic effect of the extract may also be due to the secondary metabolites presence in the extract. In conclusion the extract of *Caralluma dalzielii* possesses hypoglycemic activity which is more effective at lower doses.

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