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Effect of ethanolic extract of *Caralluma diazielli* on serum lipid profiles on fructose induced diabetes in wistar rats

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ABSTRACT

This work was conducted to evaluate the effect of ethanolic extract of *Caralluma diazielli* on lipid profiles in fructose-induced diabetes in Wistar rats. Induction of diabetes was done by dissolving 20g/100ml (20%) of fructose in distilled water. After which the animals were randomly assigned into 5 groups of 5 rats each. Group 1 as negative control received distilled water (5mg/kg), Group 2 received positive control received glibenclimide orally (1mg/kg b w), Group 3 received 200mg/kg b w of extract of *C.diazielli* orally, Group 4 received 100mg/kg b w of extract of *C.diazielli* orally Group 5 received 50mg/kg b w of extract of *C.diazielli* orally. The results obtained showed statistical significant reduction ($p < 0.05$) in the level of serum total cholesterol and triglyceride in all groups that received the extract when compared to control group. However, the serum level of high density lipoprotein was significantly increased in the groups that received the extract when compared to the negative control group. The preliminary phytochemical screening of the extract of *C.diazielli* revealed the presences of cardenolides carbohydrate flavonoid and glycosides. Also the Acute toxicity of the extract was found to be 2.154 mg/kg orally.

Key words: Fructose, *C.diazielli* lipid profiles, glibenclimide.

INTRODUCTION

Fructose a naturally found sugar in many fruit is now commonly used as industrial sweetener and is excessively consumed in western diets. High fructose intake is increasingly recognized as causative in development of pre-diabetes and metabolic syndrome [1]. The metabolic syndrome is a constellation of pathologies including obesity, insulin resistance, dislipidemia, and hypertension [2] (Elliott *et al.*, 2002). In animal studies, consumption of diets high in fructose produces obesity, insulin resistance and dyslipidemia [2,3]. Fructose induced dislipidemia also contributes to insulin resistance and glucose intolerance [4]. Dyslipidemia is common in both insulin deficiency and insulin resistance which affects enzymes and pathways of lipid metabolism. It is a well recognized risk factor for cardiovascular diseases which is currently a leading cause of morbidity and mortality worldwide [5]. Abnormalities of lipids metabolism include elevated levels of triacylglycerols (TG), total cholesterol, decreased high density lipoprotein cholesterol (HDL-C) and increased low density lipoprotein-cholesterol (LDLC), which are documented as risk factors for atherogenesis [6].

The aim of this research work was to determine the effect of ethanolic extract of *Caralluma diazielli* on Serum Lipid Profiles on Fructose induced diabetes in Wistar Rats

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 pharmacognosy and drug development, Faculty of Pharmaceutical sciences, Ahmadu Bello University, Zaria. The required quantity of the plant (*Caralluma dalzielii*) was crushed, using a pestle and mortar, and dried. It was then macerated with 70% ethanol and 30% water for 24 hours. The content was filtered and the filtrate was then poured inside an evaporating dish for concentration. This was evaporated at about 35°C and was collected, and allowed to condense.

Animals

A total of twenty five Wistar rats of both sexes between the ages of 8 to 12 weeks old and weighed between 120-150grams were used for this study. The animals were housed in the Animal House, Department of Human Physiology, ABU, Zaria, Nigeria. The animals were randomized into experimental and control groups and were kept in polypropylene cages. The animals were fed on standard feeds (Vital feeds, Jos Nigeria) and allowed access to water *ad libitum*. The "Principle of laboratory animal care" (NIH publication No 85- 23)' guideline and procedures were followed in this study (NIH publication reserved 1985).

Chemicals used

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[7,8] ; flavonoids with the use of Mg and HCl [9,10] ; tannins with 1% gelatin and 10% NaCl solutions and saponins with ability to produce suds [10].

3.4 Acute toxicity study

The lethal dose (LD₅₀) of the plant extract was determined by method of [11] Lorke 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.

Experimental design

D-Fructose (BDH, Poole, England) with a molecular weight of 180.16 was used for the study. Each rat, regardless of their weight was made diabetic by feeding them with 20% (20g/100ml) of fructose dissolved in distilled water for a period of six (6) weeks [12].

Group 1: Administered to 1ml of distilled water

Group 2: Administered to 1mg/kg b.w of glibenclimide

Group 3: Administered to 200mg/kg b w of extract *Caralluma dalzielii*.

Group 4: Administered to 100mg/kg b w of extract *Caralluma dalzielii*.

Group 5: Administered to 50mg/kg b w of extract *Caralluma dalzielii*.

Preparation of serum samples

After the last day of administration the animals were euthanized and blood samples were drawn from the heart of each by cardiac puncture into plain tubes and were allowed to clot and the serum separated by centrifugation using Denley BS400 centrifuge (England) at 3000 r p m for 15minutes and the serum collected and then subjected to biochemical assays.

Estimation of lipid profile

Assay for serum total cholesterol

The serum level of total cholesterol was quantified after enzymatic hydrolysis and oxidation of the sample as described by method of [13]. 1000µl of the reagent was added to each of the sample and standard. This was incubated for 10 minutes at 20-25 °C after mixing and the absorbance of the sample (A_{sample}) and standard (A_{standard}) was measured against the reagent blank within 30 minutes at 546nm. The value of TC present in serum was expressed in the unit of mg/dl.

Assay for serum triglyceride

The serum triglyceride level was determined after enzymatic hydrolysis of the sample with lipases as described by method of [14]. 1000µl of the reagent was added to each of the sample and standard. This was incubated for 10 minutes at 20-25 °C after mixing and the absorbance of the sample (A_{sample}) and standard (A_{standard}) was measured against the reagent blank within 30 minutes at 546nm. The value of triglyceride present in the serum was expressed in the unit of mg/dl.

Assay for serum high-density lipoprotein cholesterol

The serum level of HDL-C was measured by the method of [15]. Low-density lipoproteins (LDL and VLDL) and chylomicron fractions in the sample were precipitated quantitatively by addition of phosphotungstic acid in the presence of magnesium ions. The mixture was allowed to stand for 10 minutes at room temperature and centrifuged for 10 minutes at 4000 rpm. The supernatant represented the HDL-C fraction. The cholesterol concentration in the HDL fraction, which remained in the supernatant, was determined. The value of HDL-C was expressed in the unit of mg/dl.

Statistical analysis

All the data are expressed as mean \pm SEM. Statistical comparisons were performed by one way analysis of variance (ANOVA) followed by Duncan's multiple range tests [16]. The results were considered statistically significant if the p values were 0.05 or less. The data were analyzed using Sigma Stat v2.0 (Jandel Scientific, Palo Aho, CA, USA).

RESULTS

Table 1: Phytochemical analysis of ethanolic extract of *Caralluma dalzielii*

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

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

Acute Toxicity Study

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

Table 2: Effect of Ethanolic Extract of *Caralluma diazielli* on Serum Lipid Profiles

Treatment Given	Serum total cholesterol (mmol/L)	Serum triglyceride (mmol/L)	Serum high density lipoprotein (mmol/L)
Negative control (5ml/kg)	2.92 \pm 1.70	1.30 \pm 1.22	42.0 \pm 1.98
Positive control glibenclamide (1mg/kg)	1.72 \pm 0.83 ^a	1.00 \pm 0.88 ^a	58.4 \pm 1.23 ^{ns}
50mg/kg b.w <i>Caralluma diazielli</i>	1.94 \pm 0.77 ^a	0.62 \pm 0.36 ^a	72.4 \pm 0.96 ^a
100mg/kg b.w <i>Caralluma diazielli</i>	1.86 \pm 0.88 ^a	0.77 \pm 0.48 ^a	69.2 \pm 1.11 ^a
200mg/kg b.w <i>Caralluma diazielli</i>	1.42 \pm 0.46 ^{ns}	0.56 \pm 0.43 ^a	63.8 \pm 0.91 ^a

$P < 0.05$ is statistically significant when compared to the control group while ns= significant and ns = non significant

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 after 48 hours of extract administration.

DISCUSSION

The present study sustained hyperglycemia was achieved in animals regardless of their weight by feeding them with 20% (20g/100ml) of fructose dissolved in distilled water for a period of six weeks[12]. This finding in our current work agrees with the report of [17] who demonstrated persistent hyperglycemia in rats with features similar those seen in patients with type 2 diabetes mellitus (DM) following ingestion of high doses of fructose over a prolonged period, hence its use in type 2- like DM induction in animals. In patients with diabetes, alteration in distribution of lipid increased risk of atherosclerosis. Specifically, insulin resistance and insulin deficiency have been identified as phenotype of dyslipidemia in diabetes mellitus [18,19]. This is usually characterized by high plasma triglyceride level, low HDL cholesterol level and increased level of small dense LDL-cholesterol [20]. Abnormalities in plasma lipids and lipoprotein patterns due to defect in insulin insufficiency has been well documented, in both type I and type II diabetes mellitus [21]. In recent years, considerable interest has been directed towards the investigation of plasma lipids and lipoproteins pattern in diabetes mellitus due to the fact that abnormal lipid level leads to the development of coronary artery disease in diabetic patients [22]. Therefore, preventing or reducing the increase in serum cholesterol is associated with reducing the risk of CVD [23]. In this study, there was a statistically significant reduction ($p < 0.05$) in the total cholesterol levels of the treated groups with the extract of *Caralluma diazielli* when compared to control. The observations from the present study agree with those of [24,25] reported that the fructose-fed rat model develops an insulin-resistance syndrome with a very similar metabolic profile to the human condition, including hypertriglyceridemia, and decreased HDL cholesterol. Plasma triglycerides (TG) have been reported to increase both in humans [26,27], following the ingestion of high doses of fructose. This may be due to stimulation of hepatic de novo lipogenesis (DNL) and increased secretion of triglyceride-rich particles by the liver or to decreased extra-hepatic clearance of triglyceride particles [28,29]. In addition low HDL cholesterol and increased triglyceride levels may contribute to the increased risk of cardiovascular disease [30].

As regards to the serum triglyceride level there was a significant decrease ($p < 0.05$) in all the groups when compared to the negative control group. However, in relation to the serum high density lipoprotein there was a significant increase ($p < 0.05$) in the groups administered to the extract when compared to negative control group.

In conclusion the extract of *Caralluma diazielli* significantly reduced serum total cholesterol and triglyceride and elevated high density lipoprotein in fructose induced diabetes. The results support the traditional use of this plant in treatment of cardiovascular and diabetes. Also suggest the presence of biologically active principles which may be worth further investigation and elucidation.

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