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Effect of ethanolic extract of *Caralluma daizielii* on serum electrolytes levels on fructose- induced diabetes in wistar rats

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ABSTRACT

This aim of the experiment is to evaluate the effect of ethanolic extract of Caralluma diazielli on Serum electrolytes levels on 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 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 reductions (p<0.05) in levels of serum sodium (Na^+) and serum potassium(K^+) all the tested doses when compared with the control untreated group. However there is no statistical significant in the serum level of chloride (Cl when compared with the control untreated group. In relation to the serum level of bicarbonate (HCO₃) there was a significant decrease in the groups treated with the extract of C.diazielli when compared to control group, while there was no significant change in the group treated with the standard drug (Glibenclimide). The preliminary phytochemical screening 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: Diabetes serum electrolytes, glibenclimide, fructose

INTRODUCTION

Diabetes mellitus is a disorder in which the body is unable to metabolize carbohydrates properly. The disease is characterized by excessive amounts of sugar in the blood and urine; inadequate production and/or utilization of insulin; and by thirst, hunger and loss of weight [1]. Type 2 diabetes mellitus accounts for approximately 85 % of all cases of diabetes mellitus and is an important risk factor for cardiovascular morbidity and mortality [2]. Fructose, a natural sugar found in many fruits, is consumed in significant amounts in Western diets [3] (Miller and Adeli, 2008). Electrolyte imbalance secondary to compromise in kidney function in prolonged and uncontrolled hyperglycemia of diabetes mellitus has long been established. Usually, glycosuria, a prominent diagnostic feature of diabetes mellitus imposes dehydration via glucose osmotic diuresis, which is usually accompanied with severe loss of electrolytes including sodium, potassium, calcium, chlorine and phosphates [4]. Ingestion of high doses of fructose over a prolonged period has been used to induce persistent hyperglycaemia rats with features similar to those seen in patients with type 2 diabetes mellitus (DM), hence its use in type 2- like DM induction in animals[5].

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The aim of this research work was to determine the effect of ethanolic extract of *Caralluma diazielli* on Some Electrolytes Levels 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) has been deposited.

Extraction

The extract was prepared in the Department of pharmacognosy and drug administration, 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 [6,7]. Flavonoids with the use of Mg and HCl[8,9]. Tannins with 1% gelatin and 10% NaCl solutions and saponins with ability to produce suds [9].

3.4 Acute toxicity study

The lethal dose (LD_{50}) of the plant extract was determined by method of [10], 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 [11].

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.

Collection and preparation 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.

Tanko Y., et al

Determination of blood glucose level

All blood samples were collected from the tail vein of the rats. Fasting blood glucose levels were determined by using glucose oxidase method [12], using a digital glucometer (Accu-Chek Advantage, Roche Diagnostic, Germany) Rats having fasting blood glucose level > 180 mg/dl were considered as diabetic and used for the study

Estimation serum electrolytes

Serum sodium and potassium ions were measured by the flame photometry method of [13] and bicarbonate ion was determined using the titration method of [14], Chloride ion was analysed using the method of [15].

Statistical analysis

Data collected from the control and experimental animals were expressed as mean \pm SEM. The data were statistically analyzed using ANOVA with multiply comparisons versus control group. The values of p < 0.05 were considered significant [16].

RESULTS

Phytochemical analysis

Constituents	Inference		
1. Alkaloids	-		
2. Cardenolides	+		
Carbohydrates	+		
Flavonoides	+		
glycosides	+		
Saponins	+		
7. Tannins	+		
Triterpenes	+		
9. Steroid nucleus	-		

Table 1: Phytochemical analysis of ethanolic extract of Caralluma dalzielii

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

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 1: Effect of	of Caralluma	<i>ı daizielii</i> on	Serum	Electrolytes Levels
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Treatment Given	Na ⁺ (mmol/L)	K ⁺ (mmol/L)	Cl ⁻ (mmol/L)	HCO3 ⁻ (mmol/L)
Negative control (5mg/kg)	148.2±2.45	5.06 ± 0.14	109 ± 3.2	27.0 ± 0.70
Positive control(1mg/kg)	138.8±0.80 ^a	4.10±0.18 ^a	98.8 ± 1.01^{ns}	$23.8 \pm 0.80^{\mathrm{ns}}$
(Extract 200mg/kg b w)	139.9 ± 1.50^{a}	4.80 ± 0.27^{a}	$100.0 \pm 1.67^{\text{ns}}$	22.8 ± 1.35^{a}
(Extract 100mg/kg b w)	142.0 ± 1.62^{a}	4.20 ± 0.15^{a}	101 ± 1.32^{ns}	23.6 ± 0.74^{a}
(Extract 50mg/kg b w)	140.6±1.38ª	$4.42{\pm}0.29^{a}$	98.4 ± 0.74^{ns}	23.2±1.35ª

Values presented as mean $\pm SEM$

^{*a*} p < 0.05 is statistically significant when compared to control group while ns = non significant.

DISCUSSION

Ingestion of high doses of fructose over a prolonged period has been used to induce persistent hyperglycaemia in rats with features similar to those seen in patients with type 2 diabetes mellitus (DM), hence its use in type 2 diabetes mellitus induction in animals [5]. The method of [11] used in this study is acceptable in that there was a significant hyperglycemia following chronic ingestion of fructose. In this present study, feeding the animals with

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high doses of fructose in the laboratory for a period of six weeks resulted in progressive significant increase in blood glucose level. In this study, administration of 100mg/kg b w of vitamin C resulted to significant decrease in blood glucose level in the diabetic rats when compared to the diabetic control group. Vitamin C may play an important role in physiological reactions such as mixed function oxidation involving incorporation of oxygen into a biochemical substrate. In addition, vitamin C is considered the most important antioxidant in extracellular fluids and its antioxidant function has been shown to efficiently scavenge superoxide, hydrogen peroxide, hydroxyl, peroxyl and singlet oxygen radicals [17,18]. Therefore, the antioxidant function of vitamin C as observed in this work is related to its reversible oxidation and reduction characteristics[17]. Vitamin C has been reported to efficiently scavenge free radicals before they can initiate lipid peroxidation, and contribute to stability of cellular and basal membranes [19]. Similarly, treatment of the diabetic animals with 100mg/kg b w of vitamin E significantly reduced the blood glucose level when compared with the control group. This essential fat-soluble vitamin functions primarily as an antioxidant[20]. Our findings in this present work is in agreement with the report of [21,22] who demonstrated that there was positive effects on diabetes mellitus and improvements in glycemic control from vitamin E supplementation. The hypoglycemic effects of both vitamins are comparable to the standard drug metformin. Synergism between vitamins C and E have been demonstrated[23]. Although both vitamins serve as free radical scavengers in biological system, vitamin C is hydrophilic and exerts its effect in the extracellular space, trapping radicals in the aqueous phase [24], while vitamin E is a lipid soluble antioxidant within the cells, where the reactive metabolites are actually produced [23]. Furthermore, vitamin C interacts with tocopheroxyl radical and generates the reduced tocopherol [25]. Electrolytes play an important role in many body processes, such as controlling fluid levels, acid-base balance (pH), nerve conduction, and blood clotting and muscle contraction. Electrolyte imbalance resulting from kidney failure, dehydration, and fever and vomiting has been suggested as one of the contributing factors toward complications observed in diabetes and other endocrine disorders [26]. Diabetes is characterized by increased volume and metabolites excretions via the kidneys, usually in excess of normal thresholds. These usually give rise to derangements in homeostatic balance with respect to electrolytes [27] . In the present study treatment of fructose-induced diabetic animals with vitamin C (100mg/kg b w) and E (100 mg/kg b w) produced a significant decrease on serum sodium ion concentration; however vitamin E (100 mg/kg b w) did not produce a significant change on the level and potassium ion concentration. On other hand, administration of vitamin C (100 mg/kg b w) and E (100 mg/kg b w) recorded a non significant difference on serum chloride ion level in the diabetic animals when compared to diabetic untreated rats.

CONCLUSION

The results obtained in our study demonstrated that vitamin C and E at tested doses significantly reduced blood glucose level. However, there was a significantly decreased serum sodium ion and bio-carbonate in all groups that received Vitamin C and E. In regards to serum potassium ion, only Vitamin C at tested dose of 100mg/kg b w produced a significant change when compared to diabetic control group.

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