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Ameliorative effect of ethanolic extract of *Caralluma diazielli* on serum liver enzymes on fructose induced diabetic in wistar rats

¹Tanko Y., ¹Adamu B. Y., ¹Mohammed K. A., ¹Jimoh A., ¹Abdulrazak A., ¹Sada N. M and ²Yerima M.

¹Department of Human Physiology, Ahmadu Bello University, Zaria, Nigeria

²Department of Pharmacology and Therapeutic, Ahmadu Bello University, Zaria, Nigeria

ABSTRACT

This work was conducted to evaluate the effect of ethanolic extract of *Caralluma diazielli* on liver enzymes 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 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 showed There was a significant decrease ($p < 0.05$) levels of all serum liver enzymes (AST, ALT and ALP) in all groups administered to the extract of *C.diazielli* when compared to the negative control group. 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: Alanine transaminase ,Aspartate transaminase,Alkaline phosphatase , fructose, 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]. In animal studies, consumption of diets high in fructose produces obesity, insulin resistance and dyslipidemia [2,3].

The liver plays a major role in the regulation of carbohydrate, lipids and protein metabolism, it also has the capability to store glucose as glycogen and synthesize glucose from non carbohydrate sources through the use of it hepatocellular enzymes [4]. Increased activities of liver enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline Phosphatase (ALP) are indicators of hepatocellular injury, associated with insulin resistance [5], metabolic syndrome, and type 2 diabetes [6,7].

The aim of this research work was to determine the ameliorative effect of ethanolic extract of *Caralluma diazielli* on Serum Lipid Profiles and Liver Enzymes 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 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 [8,9] ; flavonoids with the use of Mg and HCl [10,11] tannins with 1% gelatin and 10% NaCl solutions and saponins with ability to produce suds [11].

Acute toxicity study

The lethal dose (LD₅₀) of the plant extract was determined by method of [12] 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 [13].

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.

Evaluation of serum liver enzymes

The serum enzymes Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP) were determined spectrophotometrically, using enzymatic colometric assay kits according to the laboratory procedures of Randox Laboratories Limited kits, United kingdom, using Reitman and Frankel method[14] .

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 [15] . 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 dazielii*

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

Key: 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 after 48 hours of extract administration.

Table 2: Effect of Ethanolic Extract of *Caralluma diazielli* on Serum Liver Enzymes on fructose –induced diabetes.

Treatment Given	Serum AST (U/L)	Serum ALT (U/L)	Serum ALP (U/L)
Negative control (5ml/kg)	27.8 $\pm$ 0.860	44.8 $\pm$ 3.54	82.4 $\pm$ 3.20
Positive control glibenclamide (1mg/kg)	21.4 $\pm$ 2.13 ^a	40.0 $\pm$ 1.22 ^{ns}	73.8 $\pm$ 3.02 ^{ns}
50mg/kg b.w <i>Caralluma diazielli</i>	19.4 $\pm$ 1.21 ^a	31.4 $\pm$ 2.65 ^a	72.4 $\pm$ 3.44 ^a
100mg/kg b.w <i>Caralluma diazielli</i>	20.6 $\pm$ 2.03 ^a	44.8 $\pm$ 3.31 ^a	69.2 $\pm$ 3.18 ^a
200mg/kg b.w <i>Caralluma diazielli</i>	22.4 $\pm$ 1.32 ^{ns}	39.0 $\pm$ 3.64 ^{ns}	63.8 $\pm$ 3.39 ^a

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

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[13] . This finding in our current work agrees with the report of [16] 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[17,18].

The liver is an organ of central metabolic importance and is known to undergo free radical mediated injury in diabetes mellitus[19]. Although both AST and ALT are common liver enzymes because of their higher

concentrations in hepatocytes, only ALT is remarkably specific for liver function since AST is mostly present in the myocardium, skeletal muscle, brain and kidneys[20, 21] . In general with liver disease, serum levels of AST and ALT rise and fall at the same time [22]. A mild elevation of AST level has been shown to be associated with liver injury or myocardial infarctions[23] . The higher the activity of AST, the larger the infarctions size[24]. ALT level is known to increase in liver disease and it has been used as a tool for measuring hepatic necrosis, especially in small animals [25]. In a serious liver injury through oxidative stress, currently available drugs have little effect and thus create a demand to develop new drugs. Herbs have attracted a great deal of interest as physiologically functional foods and as a source for the development of drugs because herbal constituents may have stimulating or regenerating effect on hepatocytes and restored the activities of hepatic system through their anti-hepatotoxic, antioxidant and anti hyperlipidemic activities [26].

The results of this study showed that the alkaline phosphatases, ALT and AST plasma levels were significantly decrease in fructose- induced diabetes treated with *C.diazielli* when compared to negative control. The liver plays an important role in many metabolic processes such as glycemic control, detoxification of xenobiotics, synthesis of lipoproteins, hormones and enzymes [27]. Liver enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase AST, and alkaline phosphatase (ALP) are known marker enzymes for the assessment of the functional integrity of the liver cells [28]. In diabetes mellitus, the liver is associated with abnormalities such as elevations in serum aspartate aminotransferase, alkaline phosphatase and alanine aminotransferase [29]. The present research work recorded a significantly elevated level of serum liver enzymes in the fructose-induced untreated animals. Increase in the levels of these enzymes in diabetes may be as a result of leaking out of these enzymes from the tissue into the blood stream. This is also coupled with the fact that liver tissues are grossly damaged during diabetes mellitus, Liver-alkaline phosphatase is usually mobilized most rapidly into blood and its levels in plasma may increase at early periods of liver damage [30] . In our present work there was a significantly decreased level of serum liver enzymes in all tested doses of the extract of *C.diazielli*.

CONCLUSION

The extract of *Caralluma diazielli* significantly reduced serum liver enzymes on fructose induced diabetes in Wistar rats.

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