Original Research Article

EVALUATION OF THE CARDIOPROTECTIVE EFFECT OF *CITRULLUS LANATUS* (*C. LANATUS*) SEEDS IN STREPTOZOTOCIN INDUCED DIABETIC ALBINO RATS.

ABSTRACT

This study was carried out to examine the potential of the ethanolic extract of *C. lanatus* in ameliorating cardiovascular dysfunctions in streptozotocin induced diabetic albino rats. Diabetes was induced with streptozotocin (50mg/kg) and the rats were divided into five groups - Normal control, Diabetic control, Diabetic rats treated with *C. lanatus* (100mg/kg, 400mg/kg, 800mg/kg). Analyses were carried out in two phases – at the end of two weeks (14days) and at the end of four weeks (28 days). Biochemical analysis was performed on the blood and heart tissues at the end of two weeks and four weeks. The data obtained were compared using one way analysis of variance (ANOVA) and the difference between means was obtained using Tukey's multiple comparison test. Analysis showed that creatine kinase (CK-MB) and lactate dehydrogenase (LDH) were significantly decreased ($p<0.05$) in comparison to the control. These changes were observed in a dose dependent manner. *Citrullus lanatus* showed significant potentials in maintaining integrity of the heart.

Keywords: ***Citrullus lanatus***, Diabetes, Streptozotocin, Cardioprotective, Cardiac enzymes

1. INTRODUCTION

Diabetes is a group of metabolic diseases which occurs due to a defect or a malfunction in insulin production or insulin action and is characterized by hyperglycemia. It results from impaired function in carbohydrate, protein and lipid metabolism leading to long term health complications (5)[1]. Cardiovascular complication has been highlighted as one of such health complications. The incidence of myocardial dysfunction is higher in diabetic patients as compared to non-diabetic patients (18)[2] and the mechanism underlying the development of cardiovascular dysfunction seems to be quite complex and uncertain though various hypothesis including hypoinsulinemia, deregulated carbohydrate and lipid metabolism, formation of advanced glycation end products and oxidative stress have been suggested to explain the relationship between diabetes and cardiovascular disease. Hyperglycemia which is the metabolic hallmark of diabetes inflicts cellular injury with oxidative stress playing a supportive role (19)[3]. Antioxidants acts as defense to minimize the destructive effects of the free radicals and are grouped into non enzymatic and enzymatic antioxidants. The non enzymatic antioxidants include glutathione, lycopene, vitamins like vitamins A and C, while the enzymatic antioxidants include catalase, peroxidase, and glutathione S transferase etc. *Citrullus lanatus* which is grouped amongst the curcubitaceae [Cucurbitaceae] family is rich in flavonoids, alkaloids, tannins, phenols, glycosides and these phytochemical are very useful to human health.(3)[4]. *C.lanatus* seed is said to possess antihyperlipidemic, antihyperglycemic (12)[5], antiulcerogenic, (11)[6], antioxidative and hepatoprotective properties (7)[7].

2. MATERIALS AND METHODS

2.1 Plant materials and extraction

Fresh watermelon fruits were purchased from a watermelon depot at oil-mill market, Rivers state. They were halved and the seeds collected, washed, air dried for a week and then ground into fine powder. 250g of this powdered material was macerated in 1 litre of ethanol (250g/l) for 48 hours and then filtered using a muslin cloth. The filtrate was subjected to extraction by the use of soxhlet extractor and later concentrated using the rotary evaporator.

2.2 Experimental design

Sixty albino rats (180-220g) were purchased and allowed to acclimatize for a period of two weeks during which they were fed Ad libitum on commercial dry pellet feed (top feed) and had free access to water. All animals were obtained from the Animal house, Department of Pharmacology, University of Port Harcourt and were housed in cages with saw dust bedding.

The animals had an overnight fast (about12-14 hours) after which diabetes was induced by injecting intraperitoneally, a single dose of streptozotocin (STZ) (50mg/kg) dissolved in ice cold sodium citrate buffer, (0.1M, pH 4.5). Streptozotocin is an anti microbial agent with selective cytotoxity to pancreatic β –cells that has the ability to induced a specific necrosis causing destruction of pancreatic β-cells. After 48 hours of induction, Accu-check Glucometer was used to determine the fasting blood glucose level.

Rats with glucose concentration ≥ 250mg/dl were selected for the experiment. The rats were randomly divided into five groups of each group having 12 rats. Group A (Normal control) - consisted of non diabetic rats, Group B (Diabetic control) - consisted of STZ induced diabetic rats, Group C - consisted of diabetic rats treated with 100mg/kg of *C. lanatus* seeds extract, Group D - consisted of diabetic rats treated with 400mg/kg of *C. lanatus* seeds extract, and Group E - consisted of diabetic rats treated with 800mg/kg of *C. lanatus* seeds extract.

Treatment commenced on the third day after diabetes induction. A syringe with a specially designed metal ball-ended needle was used to feed the rats by oral gavage. At the end of first 14 days from the onset of treatment with *C. lanatus* seeds extract, six of the rats were sacrificed under Diethyl ether anesthesia. This was on the 15th day after an overnight fast. Both blood and heart were collected. The remaining six continued in their treatment regime for the next 14days. At the end of the period (that is on the 29th day), the animals were sacrificed under Diethyl ether anesthesia after an overnight fast and the blood was collected by cardiac thoracic puncture of the animals. About 5ml of the blood was collected into lithium heparin (anticoagulant) bottles and the plasma was used for assay of cardiac enzymes (Creatine kinase and Lactate dehydrogenase) and the blood was centrifused at 5000 rpm 5000rpm for about 10 minutes in an automated bench top centrifuge (Hettich Universal11) and the plasma obtained was dispensed into plain bottles which were properly labeled and stored at –20^{0}C until ready for use.

2.4 Animal Ethical Compliance

During the experimental period, there was strict adherence to ethical regulations required for handling experimental animals in accordance with National and institutional guidelines for protection of Animal welfare (15)[8]

3.0 METHODOLOGY

Determination of Creatine Kinase (CK-MB) activity by Kinetic Method

Procedure

1mL of working reagent was added to 40µL of sample mixed properly and incubated at 37^{0}c for 100 seconds. The changes in absorbance per minute ($\Delta OD/\text{min}$) for 3 mins was measured.

Calculation

Creatine kinase activity (U/L) = $\Delta OD/min \times 4127$

Lactate dehydrogenase determination by kinetic method

Procedure

10µL of plasma was added to 1000µL of the working reagent and then homogenized and incubated at 37^{0}c for 1 min. The changes in absorbance per minute (Δ OD/min) during a 3 minute period was measured.

Calculation

LDH-P activity (U/L) = Δ OD/min ×1603

3.1 Statistical analysis

Values obtained were presented as mean ±standard error of mean (SEM). The statistical tool employed for analysis is one way analysis of variance (ANOVA) followed by Tukey's multiple comparison test using the Graph pad Prism Version 7.0 Software, developed by Graph Pad software, Inc, California. Values of $p<0.05$ was considered as statistically significant.

4.0 RESULTS

The results obtained from this experiment for a period of 14 and 28 days are listed in the Table 4.1 and Table 4.2.

Table 4.1: The effect of C. *lanatus* seeds extract on cardiac enzymes activity after 14 days administration

Groups/ Parameters	**CK-MB (U/L)**	**LDH (U/L)**
Group A(NC)	9.50±0.82	53.60 ±2.66
Group B (DC)	21.14±1.02	68.40± 2.14
Group C (100mg/kg)	11.11±3.27*^{ce}	64.00 ±6.83
Group D (400mg/kg)	8.44±0.71	59.40±4.53*^{bg}
Group E(800mg/kg)	7.34±1.41*^{df}	57.20±3.42*^{efh}
P-value	0.001	0.02
F – value	17.72	6.628

(*) indicated significant. Legends showing the variation in groups using Tukey test are - **a**= Group A versus Group D, **b** = Group B versus Group D, **c**= Group A versus Group C, **d**= Group A versus Group E, **e**= Group B versus Group C, **f** = Group B versus E, **g** = Group C versus Group D and **h** = Group D versus Group E, **I** = Group A versus Group B, **J**= Group C versus Group E.

Table 4.1 showed the effect of *C. lanatus* seeds extract on CK-MB and LDH levels in the rats after 14 days.

Table 4.2: The effect of C. *lanatus* seeds extract on cardiac enzymes activity after 28 days of administration.

Groups/Parameters	**CK-MB (U/L)**	**LDH(U/L)**
Group A (NC)	5.34±0.41	46.80 ±3.28
Group B (DC)	13.18±7.96*^{I}	67.20± 4.70
Group C (100mg/kg)	9.54±1.76	35.80 ± 5.00
Group D (400mg/kg)	6.68 ±0.30	24.60 ± 1.63*^{b}
Group E (800mg/kg)	6.06 ±0.62	22.60 ±2.99*^{d}

P-Value	0.007	0.0005
F - Value	4.708	8.100

(*) –significant. {Explanation for legends is needed}

Table 4.2 showed the effect of *C. lanatus* seeds extract on CK-MB and LDH levels in the rats after 28 days.

DISCUSSION

The increase in serum activities of creatine kinase and lactate dehydrogenase in experimental diabetic rats may indicate compromise on the cardiomyocytes integrity which agrees with reports that serum creatine kinase-MB (CK-MB) and lactate dehydrogenase (LDH) activities are associated with a rise in cardiac muscular damage (14)[9]. In this study there was a decrease in lactate dehydrogenase (LDH) and CK-MB activities on treatment with *C. lanatus* seed extract which would indicate that intake of these seed would be able to improve the cardiac cells integrity and may have good potentials in reducing cardiovascular risk in diabetes mellitus.

CONCLUSION

The wholesome and regular consumption of *C. lanatus* fruit (rind, pulp and seeds) should therefore be encouraged amongst diabetic and non diabetic individuals as the seeds and rind are usually discarded.

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