Original Research Article

Evaluation of the Cardioprotective Effect of *Citrullus lanatus* (*Watermelon*) Seeds in Streptozotocin Induced Diabetic AlbinoRats.

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Authors' contribution

'Author COLK' designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. 'Author ESB' and 'Author BH' managed the analyses of the study. 'Author COLK' managed the literature searches. All authors read and approved the final manuscript."

ABSTRACT

The study examined the cardioprotective potential of the ethanolic extract of *Citrullus.lanatus*in streptozotocin induced diabetic albino rats. A total of sixty albino rats weighing approximately ± 200g were used. The induction of diabetes in the rats was done using 50mg/kg body weight of streptozotocin and confirmed by checking glucose levels using glucometer. Albino rats with glucose levels greater than ≥ 250mg/dl were considered diabetic. The rats were divided into 5 groups of 12 animals each and allowed access to food and water *ad libitum*. The animals had a 12 hour overnight fast after which diabetes was induced by injecting them intraperitoneally with streptozotocin (50mg/kg). The control (group A) was not induced while diabetic control was induced. Induced diabetic rats in groups C, D and E were later treated with *C. Lantus* at a dose of 100mg/kg, 400mg/kg, 800mg/kg body weight respectively for 14 and 28 days respectively. After14days, 6 rats in each of the groups were sacrificed while the remaining 6were sacrificed after 28 days. Blood samples were collected into lithium heparin bottles and used for the assay of cardiac enzymes (creatine kinase and lactate dehydrogenase) using standard procedures. The data obtained were compared using one-way analysis of variance(ANOVA) and the difference between means was obtained using Tukey's multiple tests of comparison.Analyses showed that the levels of creatine kinase (CK-MB) and lactate dehydrogenase(LDH) in the albino rats treated with *C. lanatus*were significantly ($p<0.05$) reduced when compared with the diabetic-induced group without treatment and the decrease was dose-dependent. A significant decrease ($p<0.05$) in the levels of the enzymes was also observed based on the duration of treatment with the extract. Since the enzymes are markers of cardiac injury, the decrease in their activity following treatment with *C. lanatus*shows that the extract possesses the significant cardioprotective potential of ameliorating structural integrity of the cardiac muscle in diabetic condition.

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

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1. INTRODUCTION

Diabetes is a group of metabolic diseases which is due to a defect or a malfunction in insulin production or insulin action and is characterized by hyperglycaemia. It results from an impaired function in carbohydrate, protein and lipid metabolism leading to long term health complications[1].The cardiovascular complication has been highlighted as one of such. The incidence of myocardial dysfunction is higher in diabetic patients as compared to non-diabetic patients [1] and the mechanism underlying the development of cardiovascular dysfunction seems to look quite complex and uncertain though various hypothesis including hypoinsulinemia, deregulated carbohydrate and lipid metabolism, formation of advanced glycation end products and oxidative stress has been suggested to explain the relationship between diabetes and cardiovascular disease.*Citrullus lanatus*(water melon) which is grouped amongst the curcubitaceae family is rich in flavonoids, alkaloids, tannins, phenols, glycosides and these phytochemicals are very useful to human health[2].*C.lanatus* seed is said to possess antihyperlipidemic, antihyperglycaemic[3], antiulcerogenic[4,5], antioxidative and hepatoprotective properties[6].

2. MATERIALS AND METHODS

2.1. Plant materials and extraction

Fresh watermelon fruits were purchased from a watermelon depot at oil-mill market, Port Harcourt, Nigeria. They were halved and the seeds collected, washed,air dried for a week and then ground into fine powder. 250g of the powder was macerated in 1L of ethanol (250g/L) for 48 hours and then filtered using a muslin cloth. The filtrate was later concentrated using the rotary evaporator at a temperature of 50c.

2.2. Experimental design.

Sixty albino rats± 200g were purchased from the Animal House of the Department of Pharmacology, University of Port Harcourt, Nigeria, housed in cages with saw dust bedding 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.

The animals were fasted overnight(about12hours) after which diabetes was induced by injecting intraperitoneally with 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 –cell that has the ability to induced specific necrosis causing the destruction of pancreatic β-cells. After 48 hours of induction, Accu-check Glucometer was used to monitor the fasting blood glucose level. Rats with glucose concentration ≥ 250mg/dl were considered diabetic. The rats were randomly divided into five groups of 12 rats each. 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 groupE-consisted of diabetic rats treated with800mg/kg body weight 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 di ethyl ether anaesthesia. This was on the 15^{th} day after an overnight fast. Blood samples were collectedby cardiac thoracic puncture for the determination of creatine kinase and lactate dehydrogenase enzyme activity. Treatment was continued with the remaining six animals for the next 14days making it a total of 28 days. At the end of the period (that is on the 29^{th} day), the animals were sacrificed under di ethyl ether anaesthesia after an overnight fast. Blood samples used for biochemical analyses were also collected by cardiac thoracic puncture of the animals. About 5ml of the blood was collected into lithium heparin, separated by centrifuging the whole blood at 5000rpm for about 10 minutes in an automated bench top centrifuge (Hettich Universal11). The plasma obtained was dispensed into plain bottles which were properly labelled and stored at -20^0C until ready for use.

The lithium heparin plasmasamples were used for assay of cardiac enzymes (creatine kinase and lactate dehydrogenase).

2.4. Animal Ethical Compliance

During the experimental period, there was strict adherence to ethical regulations required for handling the experimental animal in accordance with National and Institutional Guidelines for Protection of Animal welfare[7].

3. METHODOLOGY

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

The creatine kinase activity was determined by the Kinetic Method as reported by Witt & Trendelenburg [8]. The method is based on the principle creatine kinase specifically catalyzes the transphosphorylation of ADP to ATP. NADPH is then produced through a series of coupled enzymatic reactions as seen in the equation below.The rate at which it is produced is directly proportional to the CK activity. The NADPH absorbance increase is measured per min at 340nm.

3.2. Lactate dehydrogenase determination by Kinetic Method

The Kinetic method of Vanderlinde [9] was employed in the determination of lactate dehydrogenase. Lactate dehydrogenase (LDH) an oxidoreductase catalyzes the conversion of lactate to pyruvate following the reaction scheme below.

L-Lactate + NAD^+ +H LDH Pyruvate +NADH+ H^+

The rate at which NADH is formed is directly proportional to the catalytic activity of LDH and is measured by determining the increased absorbance at 340nm

3.3. Statistical analysis

Values obtained are presented as mean ±standard error of mean (SEM).The statistical comparison of means between groups was done by one way analysis of variance (ANOVA) followed by Tukey's test of multiple comparison tests using the GraphPad Prism Version 7.0 Software, developed by Graph Pad Software, Inc, California. Values of $p<0.05$ was considered as statistically significant.

4. RESULTS

The results obtained from the experiment for the period of 14 and 28 days are listed in the tables below.

Table 1.The effect of C. *lanatus* seed extract on cardiac enzymes activity after 14 days administration

Groups/ Parameters	CK-MB (U/L)	LDH (U/L)
Group A(NC)	9.500 ± 0.82^{a}	53.60 ± 2.66^{a}
Group B (DC)	21.14 ± 1.02^{ab}	68.40 ± 2.14^{ab}
Group C (100mg/kg)	11.11 ± 3.27^{abc}	64.00 ± 6.83^{ab}
Group D (400mg/kg)	8.44 ± 0.71^{cd}	59.40 ± 4.53^{ab}
Group E(800mg/kg)	7.34 ± 1.41^{bd}	57.20 ± 3.42^{ab}
P-value	0.001	0.02
F – value	17.72	6.628

Key: NC-Normal control, DC- Diabetic control, CK-MB-Creatine kinase, LDH- Lactate dehydrogenase. Means with the same subscript are significantly ($p<0.05$) different from each other.

Table 4.1 showed the effect of *C. lanatus* extract on CK-MB and LDH levels in the rats after 14 days treatment. The results showed a significant difference ($p<0.05$) in means between the various groups involved in the study. A significant difference in means was seen between the

means of CK-MB in the normal control group (group A), diabetic control group (group B) and the group treated with 100mg/kg body weight of extract (group C). Similarly, the mean of CK-MB significantly ($p<0.05$) varied between the diabetic control group, and the groups treated with 100mg/kg and 800mg/kg body weight of the extract. Significant variations ($p<0.05$) in mean was also seen between group C and group D and groups D and E respectively.

Table 2. The effect of C. *lanatus* seed 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^{ab}	46.80 ± 3.28^{ab}
Group B (DC)	13.18 ± 1.96^{a}	31.20 ± 4.70^{a}
Group C (100mg/kg)	9.54 ± 1.76^{b}	35.80 ± 5.19^{b}
Group D (400mg/kg)	6.68 ± 0.30^{ab}	20.60 ± 1.64^{b}
Group E (800mg/kg)	6.06 ± 0.62^{ab}	22.60 ± 2.99^{b}
P-value	0.007	0.0005
F - value	4.708	8.100

Key: NC-Normal control, DC- Diabetic control, CK-MB-Creatine kinase, LDH- Lactate dehydrogenase. Means with the same letter are significantly different ($p<0.05$) from each other.

Table 2 shows the effect of *C. lanatus* seeds extract on CK-MB and LDH levels in the Albino rats after 28 days treatment. The results were expressed as mean± SEM. A significant difference ($p<0.05$) between the means of the parameters according to treatment doses was observed. However, a significant difference ($p<0.05$) in mean was seen between the mean of CK-MB in the normal control group (group 1) and the diabetic control group (group 2) and the Albino rats in group C (group that was treated with 100mg/kg body weight) of extract. Also, a significant difference ($p<0.05$) in mean was observed between the mean of LDH in the normal control group (group 1) and those that were treated with 400mg/kg (group D) and 800mg/kg body weight (group E) respectively.

The comparison of the activities of CK-MB and LDH based on duration of treatment (i.e. 14 days and 28 days) was done using the student's t-test and significant variation ($p<0.05$) was observed. The variation was also related to the increase of doses seen in group C to group E.

DISCUSSION.

Creatine kinase (CK) is an enzyme that is found in many parts of the body (muscles, brain, colon and urinary bladder). Its functions physiologically in the maintenance of an adequate store of high energy phosphorylated creatine used in the restoration of ATP levels depleted during muscle contraction. CK is made up of two subunits existing in three molecular forms namely- CK-MM, CK-MB and CK-BB. CK-MB predominates in the heart muscle and CK-MM is most prevalent in the skeletal muscle. It is found in the mitochondria and cytoplasm of skeletal muscle, cardiac muscle, brain and other visceral tissues. Its main function is in the transportation of high energy phosphate group. Serum total CK activity and CK-MB concentration rise in parallel following myocardial injury. A significant elevation in the level of CK-MB has been observed in the heart effluent during myocardial ischemia band reperfusion in isolated rat hearts[10]. Jaffe *et al.* also reported that serum CK-MB is considerably more specific for myocardial damage [11]. The present study revealed that administration of *C. lanatus*to adult male albino Wistar rats caused significant $p<0.05$ decrease in the cardiac markers (CK-MB and LDH) when compared with the animals in the streptozocin diabetic group. 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[12].

In this study there was also a decrease in lactate dehydrogenase (LDH) activities on treatment with *C.lanatus* seed extract which would indicate that intake of this seed was able to improve on cardiac cells integrity and may have good potentials in reducing cardiovascular risk in diabetes mellitus. It has been reported that the increase in serum lactate dehydrogenase activity following myocardial infarction can be sustained for 4-14 days making it a good marker for diagnosis of cardiac injury after several days of MI[13].Ethanol root bark extract of *Hippocratea africana* has also been reported to significantly ($p<0.05$) cause a decrease in the activities of cardiac markers (CK-MB and LDH)[14]. The significant $p<0.05$ decrease in the activities of CK-MB and LDH in this study may be due to the presence of phytochemicals such as cardiac glycosides and flavonoids in the seeds of *C. lanatus* [14]. Cardiac glycosides have been reported to have an inverse relationship with cardiovascular disease [15].

The comparison of the activities of the enzymes following the duration of treatment between 14 and 28 days showed that there was there was sustained significant ($p<0.05$) decrease in the activities of the CK-MB and LDH as the duration of treatment increased from 14 days to 28 days. This response was also related to the increase in the dose of *C. lanatus* administered between the groups. This is an important and significant finding in the study.

CONCLUSION

The result from the present study showed that *Citrulus lanatus* seeds contains some nutrients that are capable of improving cardiac cells integrity and may have good potentials in reducing cardiovascular risk in diabetes mellitus.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest in the publication of this paper.

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