Asian Journal of Immunology

3(1): 11-22, 2020; Article no.AJI.53370

Effects of D-Ribose-L-Cysteine on Lipid Profile, Atherogenic Index and Infertility in Streptozotocin-Induced Male Diabetic Wistar Rats

Abiodun Oluwabusola Adedeji[1,2] and Patrick Kunle Orisadiran[3*]

[1]Department of Anatomy, Olabisi Onabanjo University, Ago Iwoye, Nigeria.
[2]Department of Anatomy, Ladoke Akintola University of Technology, Ogbomoso, Nigeria.
[3]Department of Biochemistry, Ladoke Akintola University of Technology, Ogbomoso, Nigeria.

Authors' contributions

This work was carried out in collaboration between both authors. Author AOA participated in study design, data collection and interpretation, contributed to drafting/ revising of manuscript. Author PKO participated in study design, data analysis and interpretation, did literature search and drafted/edited/ revised manuscript. Both authors read and approved the final manuscript.

Article Information

Editor(s):
(1) Dr. Jaffu Othniel Chilongola, Department of Biochemistry and Molecular Biology, Kilimanjaro Christian Medical University College, Tumaini University, Tanzania.
Reviewers:
(1) Rupali Sengupta, India.
(2) Ann Nkeiruka, National Root Crops Research Institute, Nigeria.
Complete Peer review History: http://www.sdiarticle4.com/review-history/53370

Original Research Article

Received 02 November 2019
Accepted 07 January 2020
Published 20 January 2020

ABSTRACT

Aims: The present study aimed at determining the effects of D-Ribose-L-Cysteine on lipid profile, atherogenic index, and infertility in streptozotocin-induced diabetic male Wistar rats.
Methods: A total of twenty-eight adult male Wistar rats were divided into four groups of seven rats each. 1: Normal control group, 2: Diabetic control group, 3: Normal rats treated with 30 mg/kg body weight of D-Ribose-L-cysteine, 4: Diabetic rats treated with 30 mg/kg body weight of D-Ribose-L-cysteine. Group 2 and 4 were injected intraperitoneally with a single dose of streptozotocin (STZ) (65 mg/kg in 0.1 M cold citrate buffer, pH 7.5) prior to D-Ribose-L-cysteine treatment. Group 4 were subsequently administered D-Ribose-L-cysteine orally 72 hours post administration of streptozotocin, twice daily for 28 days. Parameters tested include: fasting blood and serum glucose, malondialdehyde (MDA) concentration, sperm motility, morphology and count. Testosterone (TT), follicle stimulating hormone (FSH) and luteinizing hormone (LH) were also examined.

**Corresponding author: E-mail: pkorisadiran@pgschool.lautech.edu.ng;*

Results: The results showed that diabetic rats showed weights loss, increased MDA, increase blood and serum glucose levels, elevated lipid profile, altered TT, FSH and LH as well as reduced sperm count, motility and morphology. These effects were ameliorated in diabetic rats treated with D-Ribose-L-cysteine.
Conclusion: Current study revealed that D-Ribose-L-Cysteine attenuates the oxidative stress in streptozotocin-induced diabetic rats on blood glucose, lipid profile, reproductive hormone and sperm parameters.

Keywords: D-Ribose and L-Cysteine; wistar rats; diabetic rat; sperm; hormone; streptozotocin.

1. INTRODUCTION

Diabetes mellitus is a group of metabolic disorders resulting from a deficiency in insulin secretions and/or actions [1]. This deficiency, in turn, leads to accumulation of glucose in the bloodstream with obstruction of carbohydrate, fat and protein metabolism. Disease progression results in tissue damage leading to severe diabetic complications such as impairment or loss of vision, impairment of kidney function, cardiovascular and microbial complications [2]. The world prevalence of diabetes among adults between 20 and 79 years was estimated to be 6.4%, affecting 285 million adults, in 2010, and will increase to 7.7% and 439 million adults by 2030 [3]. Out of the two types of diabetes (insulin dependent and non-insulin dependent), the incidence of non-insulin dependent diabetes mellitus is much higher than the insulin-dependent diabetes mellitus [4].

Glucose is the main oxidizable substrate in various cell types. Homeostasis is maintained in a diabetic state by the activation of catabolic pathways such as gluconeogenesis and glycogenolysis [5]. As a result, lipolysis is also increased, which trigger dyslipidemia, a risk factor for the development of atherosclerosis that affects 97 percent of diabetic patients [6,7].

Dyslipidemia is characterized by elevated levels of triacylglycerol plasma and total cholesterol, increasing the concentration of very low-density lipoproteins (VLDL-cholesterol) and low-density lipoproteins (LDL-cholesterol) and decreasing the concentration of high-density lipoproteins (HDL-cholesterol) [7,8].

In different studies by Valko et al. [9] and Schilling [10], they showed that an increase in LDL-cholesterol that occurs due to the release of oxidizing agents during the metabolic pathways activated by hyperglycemia is directly related to the development of atherosclerosis, the inflammatory process triggered by the oxidation of LDL-cholesterol. Oxidized LDLs are phagocytosed by macrophages in the subendothelial layer, thereby transforming into foam cells that contribute to the formation of atherosclerotic plaque and, consequently, atherosclerosis [6,11].

Reproductive disorders in diabetic males have been widely studied. Experimental studies by O'Neill et al. [12] and Ricci et al. [13] revealed different structural and physiological reproductive dysfunctions in cases of diabetes in males. Diabetes mellitus affects male reproductive functions at multiple levels as well as its negative effects on endocrine control of spermatogenesis and/or by impairing erection and ejaculation [14]. Ricci et al. [13] established that insulin-dependent diabetes is accompanied by reduced semen volume and decreased sperm motility and vitality. This corroborates with the report of Agbaje et al. [15] which stated that a high level of blood sugar in the bloodstream may affect sperm quality and consequently decreases male fertility potentials. Also, Joao and co. [16] confirmed high rates of infertility and poor reproductive outcomes in diabetic men compared with healthy men.

Therefore, effective control of the blood glucose level is crucial in preventing, controlling or reversing diabetic complications associated with diabetics, thus improving the quality of life in diabetic patients [17]. A study has shown that antioxidant substances can reduce chronic degenerative diseases such as diabetes mellitus and its complications [18]. Antioxidants are stable substances that act as reducing agents capable of reducing oxidized substances and making them stable, thus repairing the structure of the cellular macromolecules [19].

D-ribose is an antioxidant and a prodrug form of L-cysteine known to aid the elevation of intracellular levels of glutathione (GSH) [20]. GSH is a coenzyme that mediates the protection against free radicals generated during the

oxidative metabolism of acetaminophen by the hepatic cytochrome P-450 system [21]. Whole glutathione consumption cannot be effective because it would be destroyed in the digestion process before reaching the cell. The ribose component of the D-Ribose-L-Cysteine solves these challenges allowing the cells to produce glutathione when needed by effectively protecting and delivering the fragile cysteine molecule [22].

In view of the discussed above, this study aimed to determine the beneficial effects of D-Ribose-L-Cysteine on lipid profile, atherogenic index, and infertility in streptozotocin-induced diabetic male Wistar rats.

2. MATERIALS AND METHODS

2.1 Chemicals

Streptozotocin (STZ) was a product of Sigma Chemical Company St. Louis U.S.A. All assay kits were from Randox Laboratories Limited UK. Chemicals and reagents used were of analytical grade. D-Ribose-L-Cysteine supplement was obtained from Max International, Salt Lake City, Utah, USA.

2.2 Experimental Design

A total of 28 Albino rats of Wistar strain, with an average weight of 160 g were obtained from the Animal House of Physiology Department, Ladoke Akintola University of Technology, Nigeria. The animals were divided into 4 groups of 7 rats each and housed in separate cages in the same environment. The animals were allowed to acclimatize in the laboratory for two weeks before the commencement of the experiments.

2.3 Diabetes Induction

Streptozotocin (65 mg/kg in 0.1 M cold citrate buffer, pH 7.5) was administered to Wistar rats fasted overnight by interperitoneally injection of freshly prepared solution. The animals were considered as diabetic if the blood glucose values of the overnight fasted rats, were > 250 mg/dl on the third day.

Group 1: Control rats given citrate buffer only (0.01 M, pH 7.5),

Group 2: Diabetic control (untreated rats)

Group 3: Rats treated with 30 mg/kg body weight of D-Ribose-L-cysteine.

Group 4: Diabetic rats treated with 30 mg/kg body weight of D-Ribose-L-cysteine

The diabetic rats were then kept for 24 hours on 5% glucose solution bottles in their cages to prevent hypoglycemia. Blood was collected every 3 days through the rat's tail vein for glucose estimation using One Touch Glucometer; After 28 days of treatment, the body weight and fasting blood glucose, serum glucose of the animals were again determined.

2.4 Collection of Blood

The rats were fasted overnight after 28 day, anaesthetized with chloroform and sacrificed. Blood was collected by cardiac puncture into plain sample bottles, allowed to coagulate and then centrifuged at 3000 rpm for 15 minutes to obtain serum. The serum was kept under refrigeration at 4ºC for biochemical examination.

2.5 Biochemical Examination

Serum total cholesterol was determined using the method of Zak [23], plasma triglyceride estimated by the method of Mendez et al. [24], HDL-C by Lopez- Vitrella et al. [25], LDL-C and VLDL triglyceride values were calculated by the modified method of Friedewald formula [26]. Serum MDA was measured by a thiobarbituric acid assay procedure [27].

2.6 Semen Analysis

The caudal epididymis of the rats was incised and a drop of epididymal fluid was smeared onto a glass slide, covered by a 22 · 22 mm. The slide was examined under the light microscope at 100 magnification to evaluate different fields [28]. After assessing different microscopic fields, the relative percentage of motile sperm was estimated and reported to the nearest 5% using the subjective determination of motility [29]. The sperm count was determined using the Neubauer improved hemocytometer. The epididymal fluid ratio of 1:20 was prepared by adding 0.1 ml of fluid to 1.9 ml of water. The dilution was mixed thoroughly and both sides of the counting chamber were scored and the average was taken. Spermatozoa within five of the red blood cell squares including those which lie across the outermost lines at the top and right sides were counted, while those at the bottom and left sides were left out. The number of spermatozoa counted was expressed in millions/ml [30].

2.7 Reproductive Hormones

The serum levels of testosterone, luteinizing hormone (LH) and Follicle-stimulating hormone (FSH) were measured using enzyme-linked immunoassay kit (Abcam) according to the manufacturer's instructions.

2.8 Statistical Analysis

Results were presented as Mean ± SD. Paired Student's t-test was used to compare variations amongst groups. The minimum level of significance was considered at $p<0.05$. Statistical analysis was carried out using a software program (GraphPad Prism Ver. 5; GraphPad Software, San Diego, CA).

3. RESULTS

3.1 Effect of D-Ribose-L-Cysteine on Bodyweight and Serum Glucose on STZ Induced Diabetic Rats

There was a significant increased ($p<0.05$) in the bodyweight of normal control and D-Ribose-L-Cysteine treated group whereas the body weight of diabetic rats significantly reduced during the period of the experiment (Fig. 1). The blood glucose levels increased significantly ($p < 0.05$) after the induction of diabetes but after the administration of D-Ribose-L-Cysteine the blood glucose levels also decreased significantly ($p < 0.05$) compared to the diabetic control (Fig. 2a).

The serum glucose level in the diabetic control group (15.5±1.3 mmol/L) was significantly ($P<0.05$) higher compared to normal control group (6.4±1.3 mmol/L), while treated diabetic rats showed a slight significant decreased ($P<0.05$) in the serum glucose (9.7±1.2 mmol/L) compared to the diabetic control rats (Fig. 2b).

3.2 Effect of D-Ribose-L-Cysteine on Lipid Peroxidation in STZ Induced Diabetic Rats

Figure 3 shows the changes in serum MDA concentration. The mean value of serum MDA levels in the diabetic rats (7.77±0.78 nmol/ml) significantly increased ($p<0.05$) when compared with the normal control (5.7±0.90 nmol/ml) but significantly decreased ($p<0.05$) in diabetic rats treated with D-Ribose-L-Cysteine (5.93±0.89 nmol/ml).

3.3 Effect of D-Ribose-L-Cysteine on Lipid Profile in STZ Induced Diabetic Rats

Serum levels of normal and diabetic rats treated with D-Ribose-L-Cysteine are shown in Figure 4. Diabetic control rats exhibited higher serum Total cholesterol, LDL-cholesterol, triglycerides, and low HDL-cholesterol levels compared to those of normal rats. The total cholesterol, LDL-cholesterol and triglycerides levels were significantly higher ($p<0.05$) in diabetic control rats compared to the D-Ribose-L-Cysteine treated rats (97.50±10.50, 45.23±12.34 and 8 5.49±13.80 mg/dL), D-Ribose-L-Cysteine treated diabetic rats (105.20±7.30, 33.67±12.54 and 92.62±14.30 mg/dL), and normal control rats (110.50±7.80, 43.98±15.23 and 89.407.80, mg/dL) respectively. Whereas LDL-cholesterol levels of rats treated with D-Ribose-L-Cysteine are not significantly different when compared with the normal control rats.

3.4 Effect of D-Ribose-L-Cysteine on Sperm Parameters in STZ Induced Diabetic Rats

The mean percentage of sperm motility after 28 days of treatment is shown in Figure 5. Sperm motility in the diabetic control group (35.3±7.5%) was significantly lower ($p<0.05$) when compared to the normal control group (87.7±9.1%). Treatment of diabetic group with D-Ribose-L-Cysteine produced a more pronounced effect with an increased in sperm motility (69.5 ±9.6%). The movement of sperm in D-Ribose-L-Cysteine treated group were similar to the normal control group (Fig. 5).

As shown in Figure 5, Streptozotocin-induced diabetic control group showed a significant decreased ($p<0.05$) in sperm morphology (37.6±6.3%) as compared with the normal control group (84.2±6.9). There was significantly increased ($p<0.05$) in mean sperm morphology in D-Ribose-L-Cysteine treated group and D-Ribose-L-Cysteine treated diabetic group (85.90 ± 7.00 and 70.0±10.1) in comparison with the diabetic control group (Fig. 5).

There was a significant decreased in sperm count value in the diabetic control group (33.5± 7.50, $p<0.05$) when compared with normal control group ((86.8± 7.70), D-Ribose-L-Cysteine treated rats (93.7±7.00) and D-Ribose-L-

Cysteine treated diabetic rats (75.5± 7.11.8) (Fig. 5).

3.5 Effect of D-Ribose-L-Cysteine on Reproductive Hormones in STZ Induced Diabetic Rats

Testosterone levels decreased significantly ($p < 0.05$) in diabetic group compared to non-diabetic normal control. There was no significant difference in testosterone levels of animals that received D-Ribose-L-Cysteine compared with normal control, but it increased significantly ($p < 0.05$) compared to diabetic control group (Fig. 6).

There was no significant difference in FSH of normal control and D-Ribose-L-Cysteine groups; however, there was a significant increase of FSH in animals that received D-Ribose-L-Cysteine group and D-Ribose-L-Cysteine diabetic group compared to diabetic group (Fig. 6).

Luteinizing hormone levels increased significantly ($p < 0.05$) in the D-Ribose-L-Cysteine group compared to diabetic control groups. However, diabetic animals administered D-Ribose-L-Cysteine had significantly decreased ($p < 0.05$) levels of LH compared to normal control. FSH decreased significantly ($p < 0.05$) in the diabetic control group and compared with non-diabetic normal control (Fig. 6).

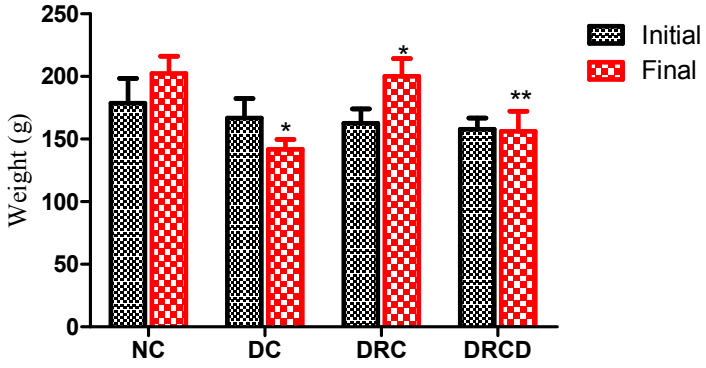


Figure 1: Effect of D-Ribose-L-Cysteine on Body weight in STZ induced diabetic rats.
Values are expressed as Mean ± SD, * significantly different from normal control group ($p<0.05$).
** Significantly different from normal and diabetic controls ($p<0.05$).
NC: Normal Control, DC: Diabetic control, DRC: D-Ribose-L-Cysteine treated rats, DRCD: D-Ribose-L-Cysteine treated diabetic rats

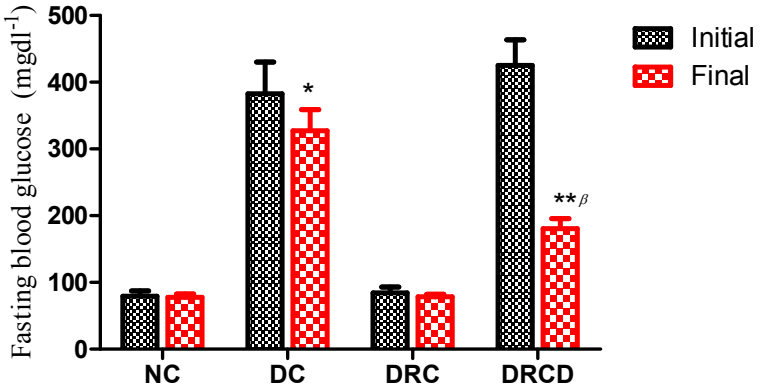


Figure 2a: Effect of D-Ribose-L-Cysteine on blood glucose in STZ induced diabetic rats.
Values are expressed as Mean ± SD, * significantly different from normal control group ($p<0.05$).
** Significantly different from normal and diabetic controls ($p<0.05$). β Significantly different from Initial Fasting Blood glucose ($p<0.05$)
NC: Normal Control, DC: Diabetic control, DRC: D-Ribose-L-Cysteine treated rats, DRCD: D-Ribose-L-Cysteine treated diabetic rats

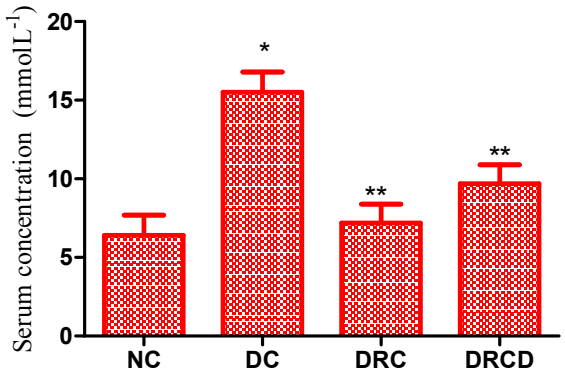
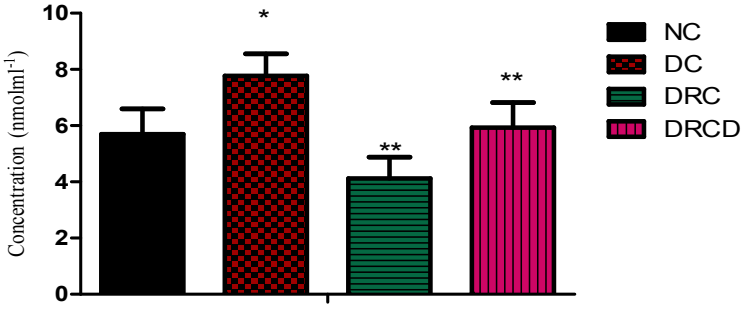


Figure 2b: Effect of D-Ribose-L-Cysteine on serum glucose in STZ induced diabetic rats.
Values are expressed as Mean ± SD, * significantly different from normal control group ($p<0.05$).
** Significantly different from normal and diabetic controls ($p<0.05$).
NC: Normal Control, DC: Diabetic control, DRC: D-Ribose-L-Cysteine treated rats, DRCD: D-Ribose-L-Cysteine treated diabetic rats



MDA

Figure 3: Effect of D-Ribose-L-Cysteine on lipid peroxidation in STZ induced diabetic rats.
Values are expressed as Mean ± SD, * significantly different from normal control group ($p<0.05$).
** Significantly different from normal and diabetic controls ($p<0.05$). NC: Normal Control,
DC: Diabetic control, DRC: D-Ribose-L-Cysteine treated rats, DRCD: D-Ribose-L-Cysteine treated diabetic rats

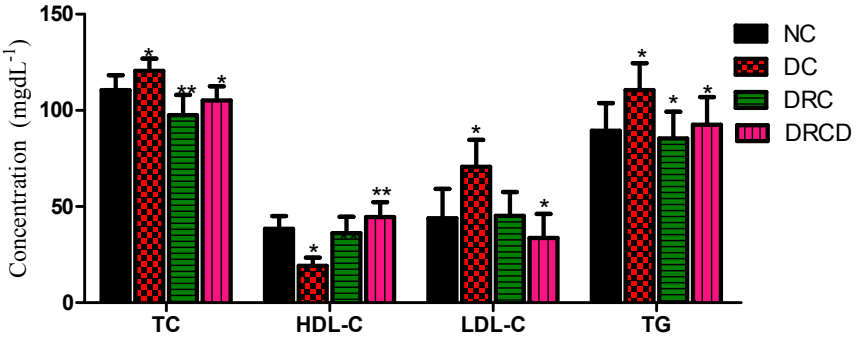


Figure 4: Effect of D-Ribose-L-Cysteine on lipid profile in STZ induced diabetic rats.
Values are expressed as Mean ± SD, * significantly different from normal control group ($p<0.05$).
** Significantly different from normal and diabetic controls ($p<0.05$). NC: Normal Control,
DC: Diabetic control, DRC: D-Ribose-L-Cysteine treated rats, DRCD: D-Ribose-L-Cysteine treated diabetic rats.
TC: Total Cholesterol, HDL-C: High density lipoprotein cholesterol, LDL-C: Low density lipoprotein cholesterol
TG: Triglycerides

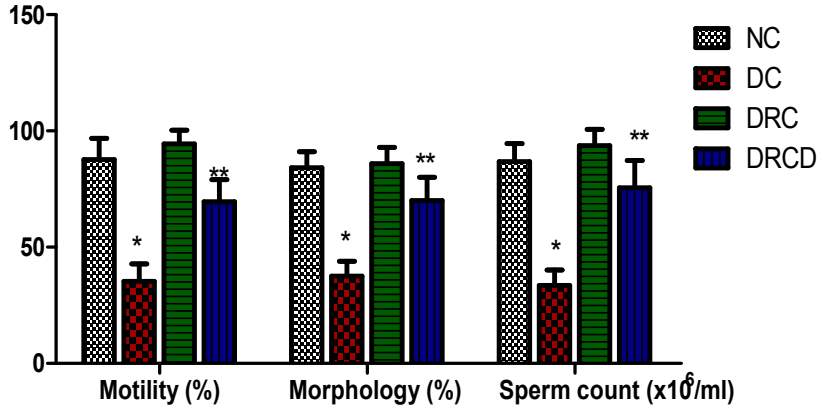


Figure 5:Effect of D-Ribose-L-Cysteine on Sperm parameters in STZ induced diabetic rats.
Values are expressed as Mean ± SD, * significantly different from normal control group ($p<0.05$).
** Significantly different from normal and diabetic controls ($p<0.05$). NC: Normal Control,
DC: Diabetic control, DRC: D-Ribose-L-Cysteine treated rats, DRCD: D-Ribose-L-Cysteine treated diabetic rats

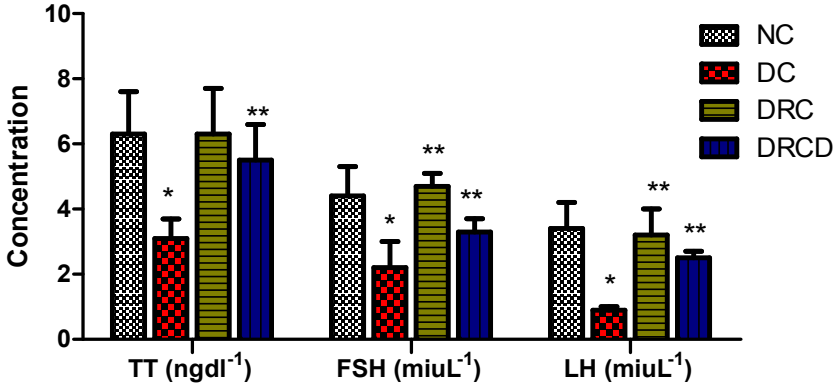


Figure 6: Effect of D-Ribose-L-Cysteine on reproductive hormone in STZ induced diabetic rats.
Values are expressed as Mean ± SD, * significantly different from normal control group ($p<0.05$).
** Significantly different from normal and diabetic controls ($p<0.05$). NC: Normal Control,
DC: Diabetic control, DRC: D-Ribose-L-Cysteine treated rats, DRCD: D-Ribose-L-Cysteine treated diabetic rats.
TT: Testosterone, FSH: Follicle stimulating hormone, LH: Lutenizing hormone

4. DISCUSSION

Diabetes mellitus is a chronic metabolic disease with debilitating complications. Two types are mostly described in the literature; type 1(insulin-dependent diabetes mellitus) and type-2 (insulin-resistant diabetes mellitus) [1]. These two interestingly, though have distinct pathogenesis, share similar life-threatening complications such as long-term hyperglycemia, which is associated with many other complications, including male reproductive dysfunctions and infertility [29]. Over 90% of diabetic patients are known to suffer from severe insulin resistance, which leads to severe metabolic and reproductive complications [30,31].

Hwang et al. [32] and Chen et al. [33] in a different study reported the toxic effects of STZ on pancreatic beta cells. These effects lead to a decrease in insulin production as well as less cellular absorption of glucose resulting in glucose toxicity, increased ROS and beta cells apoptosis. Hence, the usage of chemical substances that will aid the cellular sensitivity to insulin consequently ameliorate and prevent glucose toxicity [34]. The present study aims to investigate the ameliorative effect of D-Ribose-L-Cysteine on the blood glucose, lipid peroxidation, lipid profile and reproductive system in adult male Wistar rats.

Kalaiarasi and Pugalendi [35] reported a severe loss in body weight in streptozotocin-induced diabetes. The decrease in the bodyweight of diabetic rats in this study was due to the cytotoxic effects of streptozotocin on cells leading to loss or degradation of structural

proteins to provide amino acids for gluconeogenesis during insulin deficiency resulting to muscle wasting and weight loss. The protein content is decreased in muscular tissue by proteolysis due to insulin deficiency [36]. In the present study, diabetic control rats showed a marked reduction in their body weights when compared to normal control rats. The weight loss was reversed by the administration of D-Ribose-L-Cysteine to the diabetic rats. In addition, glucose reacts with proteins in a non-enzymatic manner leading to the development of Amadori products followed by the formation of advanced glycation end-products AGEs; ROS is generated at multiple steps during this process [37]. Elevated lipid peroxidation in tissues will result in a concomitant decrease in body weights [38] as seen in this study. D-Ribose-L-Cysteine administered maintained the body weights of the animals protecting them from the cytotoxic effects of streptozotocin. These results suggest a possibility of D-Ribose-L-Cysteine to either improve pancreatic beta cells function or prevent lipid peroxidation by impairing the formation of ROS or increasing the production of antioxidants to neutralize ROS generated. Increased oxidative stress and changes in the antioxidant capacity as observed in this study have been implicated in the etiology of chronic diabetes complications as reported by Van et al. [39] and Omotayo et al. [38].

This study demonstrated that streptozotocin increased lipid peroxidation. The elevated levels of oxidative stress markers have been associated with hyperglycemia which is due to the shortfall in insulin as a result of beta cell dysfunction [40,41]. This anomaly has been associated with complications including cardiovascular diseases in diabetes mellitus as reported by Omotayo et al. [38] and George et al. [42]. These negative effects of streptozotocin were ameliorated by D-Ribose-L-Cysteine. Since streptozotocin is known to destroy pancreatic beta cells leading to a concomitant increase in glucose availability- hyperglycemia [43], therefore, the possible mechanism of action of D-Ribose-L-Cysteine is mediated through influencing glucose uptake or utilization by tissues and probably regeneration of beta cells. Hyperglycemia may result in glucose toxicity and increase in ROS activity as well as increased lipid peroxidation especially in the pancreas with low antioxidants level [44]. This is in tandem with the increased serum MDA levels of the diabetic controls rats in this study. Furthermore, the decreased MDA levels as seen in animals that received D-Ribose-L-Cysteine showed an abrogation of cellular redox.

In this study, there was an increased in total cholesterol, LDL-cholesterol, triacylglycerides, and low HDL-cholesterol in diabetic rats, confirming the development of dyslipidemia which is supported by previous studies [45,46]. Insulin deficiency in diabetes mellitus results in increased lipolysis and subsequent β-oxidation of acetyl-CoA, a key enzyme in cholesterol biosynthesis used in lipogenesis together with HMG-CoA reductase [46], thereby promoting the hepatic formation of VLDL-cholesterol and consequently increasing serum levels of cholesterol and LDL-cholesterol. Fatty acids that are not β-oxidated are esterified into triacylglycerols which are incorporated into VLDL-cholesterol in the liver and exported to the bloodstream [47]. These metabolic events increased lipoproteins to a value above normal in the present study, identifying dyslipidemia as a risk factor for the development of atherosclerosis [46]. Improved dyslipidemia in diabetic rats treated with D-Ribose-L-Cysteine can be explained by the greater insulin secretion in the presence of this antioxidant, D-Ribose-L-Cysteine, since insulin decreases blood sugar levels and lipolysis in adipose tissue. Studies have shown that D-Ribose-L-Cysteine increases insulin secretion. On the other hand, Ayyasamy and Leelavinothan [46] revealed that insulin increases the activity of lipoprotein lipase, which catalyzes the breakdown of triacylglycerol ester bonds thereby increasing the clearance of VLDL-cholesterol.

Also, the increased insulin level as seen in diabetic control rats elevates the activity of lecithin cholesterol acyltransferase and the enzyme responsible for extracellular cholesterol esterification, thus increasing the efficiency of reverse cholesterol transport, indicating an inverse correlation with cardiovascular accidents [48,46]. Studies have identified a relationship between antioxidants and reduced cholesterol levels, due to inhibition of HMG-CoA reductase activity and cholesterol biosynthesis [49,46]. Thus, the D-Ribose-L-Cysteine administered in diabetic rats was able to normalize the atherogenic index because it was possible to control the lipid profile, a finding that is corroborated by Yang et al. [50], who reported that an increased atherogenic index is related to low antioxidant activity. Thus, D-Ribose-L-Cysteine was able to reduce the formation of atherosclerotic plaque by lowering blood glucose

levels, the glycation of LDL-cholesterol, and its consequent oxidation in the present study.

The increased antioxidant levels by D-Ribose-L-Cysteine in diabetic rats enhanced the production of FSH and LH by the anterior pituitary gland [51] which promotes follicle development and testosterone synthesis. Administration of D-Ribose-L-Cysteine in the study was found to improve the percentage sperm motility and sperm count. The antioxidant activity of this substance may be attributed to the reduction or amelioration of oxidative-stress induced diabetic complications such as lipid peroxidation, by elevation of antioxidant enzyme activities as observed from this study. In addition, previous studies involving treatments with antioxidant compounds demonstrate their importance in regulating β- pancreatic cell functions and growth, thereby reducing the complications due to diabetes [52,53].

The antioxidant activities of D-Ribose-L-Cysteine may also be responsible for the restoration of β-cells' integrity and metabolic functions, while at the same time ensuring maximum synthesis of insulin by these cells necessary for glucose tolerance [52]. Therefore, helps to reverse the reproductive dysfunctions associated with diabetes as seen in the present study [54,55]. This study further strengthens previous findings by Ballester et al*.* [56] and Suthagar et al*.* [57] that intraperitoneal administration of high doses of alloxan in male rats induces type-1 diabetogenic conditions, which leads to reproductive complications such as reduced testicular and epididymal weights, decreased testosterone production, reduced sperm motility and sperm counts, and also decrease in the gonadal function of both leydig (testosterone producing) cells and sertoli (supporting) cells.

5. CONCLUSION

In conclusion, D-Ribose-L-Cysteine therefore, revealed in the current study to attenuate the oxidative stress in streptozotocin-induced diabetic rats on blood glucose, lipid profile, spermatogenesis, and steroidogenesis. D-Ribose-L-Cysteine could be used as adjuvant therapy for the reduction of atherosclerosis and infertility in diabetic subject because of its potent antioxidant property.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The protocol of the study was approved by the Local Ethical Committee for animal experimentation of the Faculty of Basic Medical Sciences of Olabisi Onabanjo University, Nigeria.

ACKNOWLEDGEMENT

The authors especially thank Mr. Akinrinola and Sharphor Enterprises for their support during the course of this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. American Diabetes Association, diagnosis and classification of diabetes mellitus, Diabetes Care. 2014;37:S81–S90.
2. Bastaki S. Review: Diabetes mellitus and its treatment. Intl J Diabetes Metab. 2005; 13:111-34.
3. Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res Clin Pract. 2010;87:4-14.
4. Hussain AMHE. Hypoglycemic, hypolipidemic and antioxidant properties of combination of Cucurmin from *Cucurma longa Linn*. and partially purified product from *Abroma augusta Linn*. in streptozotocin induced diabetes. Indian J Clin Biochem. 2002;17:33-43.
5. Hebert SL, Nair KS. Protein and energy metabolism in type 1 diabetes. Clinical Nutrition. 2010;29(1):13–17.
6. Dokken BB. The pathophysiology of cardiovascular disease and diabetes: Beyond blood pressure and lipids. Diabetes Spectrum. 2008;21(3):160–165.
7. Kim MS, Wang Y, Rodrigues B. Lipoprotein lipase mediated fatty acid delivery and its impact in diabetic cardiomyopathy. Biochimica et Biophysica Acta (BBA) – Molecular and Cell Biology of Lipids. 2012;1821(5):800–808.
8. Grundy MS, Benjamin IJ, Burke GL Diabetes and cardiovascular disease: A statement for healthcare professionals from the american heart association. *Circulation*. 1999;100(10):1134–1146.
9. Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazur M, Telser J. Free radicals and

antioxidants in normal physiological functions and human disease. The International Journal of Biochemistry & Cell Biology. 2007;39(1):44–84.
10. Schilling JD. The mitochondria in diabetic heart failure: From pathogenesis to therapeutic promise. Antioxidants & Redox Signaling. 2015;22(17):1515–1526.
11. Schwartz EA, Reaven PD. Lipolysis of triglyceriderich lipoproteins, vascular inflammation, and atherosclerosis. Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids. 2012;1821(5):858–866.
12. O'Neill J, Czerwiec A, Agbaje I, Glenn J, Stitt A, McClure N. Differences in mouse models of diabetes mellitus in studies of male reproduction. Int J Androl. 2010;33: 709-716.
13. Ricci G, Catizone A, Esposito R, Pisanti FA, Vietri MT, Galdieri M. Diabetic rat testes: Morpholigical and functional alterations. Andrologia. 2009;41:361-368.
14. Agbaje IM, Rogers DA, McVicar CM, McClure N, Atkinson AB, Mallidis C. Insulin dependent diabetes mellitus: implications for male reproductive function. Hum Reprod. 2007;22:1871-1877.
15. Joao RS, Amaral S, Oliveira P. Diabetes and the impairment of reproductive function: possible role of mitochondria and reactive species. Bentham Science. 2009; 4:1573-3993.
16. Xie JT, Wanq A, Mehendale S, Wu J, Aung HH, Dey L, Anti-diabetic effects of *Gymnema yunnanense* extract. Pharmacol Res. 2003;47:323-29.
17. Arikawe AP, Daramola AO, Odofin AO, Obika LF. Alloxan-induced and insulin resistant diabetes mellitus affect semen parameters and impair spermatogenesis in male Rats. Reprod Health. 2006;10:106-113.
18. La Vignera S, Condorelli R, Vicari E, D'Agata R, Calogero AE. Diabetes and sperm parameters: A brief review. J Androl 2011;33:145-153.
19. Nagasawa, HT. Method to enhance delivery of glutathione and ATP levels in cells. Google Patents; 2016.
20. Roberts JC, Nagasawa HT, Zera RT, Fricke RF, Goon DJ. Prodrugs of L-cysteine as protective agents against acetaminophen-induced hepatotoxicity. 2-(Polyhydroxyalkyl)-and2-(polyacetoxyalkyl) thiazolidine-4 (R)-carboxylic acids, J. Med. Chem. 1987;30:1891–1896.
21. Benedict F, Opeyemi A, Mulikat O, Abraham O, Adeoye O. Effect of D-ribose-L-cysteine on aluminum induced testicular damage in male Sprague-Dawley rats. JBRA Assisted Reproduction. 2017;21(2): 94-100.
22. Albro PW, Corbelt JT, Schroeder JL. Application of the thiobarbiturate assay to the measurement of lipid products in microsomes. Chem Biol Interact. 1986; 86(3):185-194.
23. Zak B. Determination of total cholesterol using reaction with ferric chloride and sulphuric acid. Am J Clin Path. 1959;27: 583-590.
24. Mendez A, Franklein J, Slahegan BH. Simple manual method for determination of serum triglycerides. Clin Chem. 1975; 21(6):760-770.
25. Lopez-Vitrella MF, Stone P, Ellis S, Coltwell JA. Cholesterol determination in high density lipoprotein, separated by three different methods. Clin Chem. 1977; 23(5):882-884.
26. Sandkamp M, Funke H, Schulter M, Kahlar E, Assman G. Lipoprotein(a) is an independent risk factor for myocardial infarction at a young age. Clin Chem. 1990;36(1):20-23.
27. World Health Organization. laboratory manual for the examination of human semen and sperm cervical mucus interaction. Cambridge: Cambridge University Press, UK; 1999.
28. Keel BA, Webster BW. Handbook of the laboratory diagnosis and treatment of infertility. Boca Raton: CRC Press Incorporation. 1990;37.
29. Bartak V, Josiko M, Horackova M. Human diabetes and sperm quality. Int. J. Fertil. 1975;20:30-32.
30. Faerman I, Vilar O, Riverola M, Rosner J, Jadzinsky M, Fox D, Perez A, Ber nstein-Hahn L. Impotence and diabetes. Studies of androgenic function indiabetic impotent males. Diabetes. 1972;21:23-30.
31. Schloffing K. Hypogonadism in male diabetic subjects. In: On the nature and treatment of diabetes. Leibel B, Wrenshall G, eds. Excerpta Medica. 1965;505-521.
32. Hwang W, Bak D, Kim D, Hong J, Han S, Park K, Lim K, Lim D. Attenuation of streptozotocin-induced pancreatic beta cell death in transgenic fat-1 mice via

autophagy activation, Endocrinol. Metab. (Seoul, Korea); 2015.
33. Chen WB, Gao L, Wang J, Wang YG, Dong Z, Zhao J, Mi QS, Zhou L, Conditional ablation of HDAC3 in islet beta cells results in glucose intolerance and enhanced susceptibility to STZ-induced diabetes, Oncotarget. 2016;7:57485.
34. Tang C, Ahmed K, Gille A, Lu S, Gröne HJ, Tunaru S, Offermanns S. Loss of FFA2 and FFA3 increases insulin secretion and improves glucose tolerance in type 2 diabetes, Nat. Med. 2015;21: 173.
35. Kalaiarasi P, Pugalendi KV. Antihyperglycemic effect of 18β- glycyrrhetinic acid, a glycone of glycyrrhizin, on streptozotocin diabetic rats. Eur J Pharmacol. 2009; 606:269-73.
36. Babu SP, Prabuseenivasan P, Ignacimuthu S. Cinnamaldehyde- A potential antidiabetic agent. Phytomedicine. 2007;4:15-22.
37. Karasu C. Glycoxidative stress and cardiovascular complications in experimentally induced diabetes: effects of antioxidant treatment, Open Cardiovasc. Med. J. 2010;4.
38. Omotayo EO, Gurtu S, Sulaiman AS, Wahab MSA, Sirajudeen K, Salleh MSM. Hypoglycemic and antioxidant effects of honey supplementation in streptozotocin-induced diabetic rats, Int. J. Vitam. Nutr. Res. 2010;80:74.
39. Van Dam PS, Van Asbeck BS, Bravenboer B, Van Oirschot JF, Gispen WJ, Marx JJ. Nerve function and oxidative stress in diabetic and vitamin E-deficient rats, Free Radic. Biol. Med. 1998;24:18–26.
40. Macut D, Bjekić-Macut J, Savić-Radojević A. Dyslipidemia and oxidative stress in PCOS, Polycystic Ovary Syndrome, Karger Publishers; 2013.
41. Tangvarasittichai S. Oxidative stress, insulin resistance, dyslipidemia and type 2 diabetes mellitus, World J. Diabetes. 2015; 6:456–480.
42. George N, Kumar TP, Antony S, Jayanarayanan S, Paulose C. Effect of vitamin D 3 in reducing metabolic and oxidative stress in the liver of streptozotocin-induced diabetic rats, Br. J. Nutr. 2012;108:1410–1418.
43. Lenzen S, The mechanisms of alloxan-and streptozotocin - induced diabetes, Diabetologia. 2008;51:216–226.
44. Li L, Leung PS. Pancreatic cancer, pancreatitis, and oxidative stress. gastrointestinal tissue, Elsevier; 2017.
45. Almeida DAT, Braga CP, Novelli ELP, Fernandes AAH. Evaluation of lipid profile and oxidative stress in STZ-induced rats treated with antioxidant vitamin. Brazilian Archives of Biology and Technology. 2012; 55(4):527–536.
46. Ayyasamy R, Leelavinothan P. Myrtenal alleviates hyperglycaemia, hyperlipidaemia and improves pancreatic insulin level in STZ-induced diabetic rats. Pharmaceutical Biology. 2016:54(11):2521–2527.
47. Kim MS, Wang Y, Rodrigues B. Lipoprotein lipase mediated fatty acid delivery and its impact in diabetic cardiomyopathy. Biochimica et Biophysica Acta (BBA) – Molecular and Cell Biology of Lipids. 2012;1821(5):800–808.
48. Dokken BB. The pathophysiology of cardiovascular disease and diabetes: beyond blood pressure and lipids. Diabetes Spectrum. 2008;21(3):160–165.
49. Pereira Braga C, Momentti AC, Barbosa Peixoto F. Influence of treatment with quercetin on lipid parameters and oxidative stress of pregnant diabetic rats. Canadian Journal of Physiology and Pharmacology 2013;91(2):171–177.
50. Yang R, Le G, Li A, Zheng J, Shi Y. Effect of antioxidant capacity on blood lipid metabolism and lipoprotein lipase activity of rats fed a high-fat diet. Nutrition Journal. 2006;22(11-12):1185–1191.
51. Jiang X, Chu Q, Li L, Qin L, Hao J, Kou L, Lin F, Wang D. The anti‐fatigue activities of Tuber melanosporum in a mouse model, Exp. Ther. Med. 2018;15:3066–3073.
52. Szudelski T. The mechanism of alloxan and streptozotocin action in b-cells of the rat pancreas. Physiol. Res. 2011;50:536-546.
53. Coskun O, Kanter M, Korkmaz A, Oter S. Quercetin, a flavonoid antioxidant, prevents and protects streptozotoc ininduced oxidative stress and cell damage in rat pancreas. Pharmacol. Res. 2005; 51:117-123.
54. Wankeu-Nya M, Florea A, Bâlici S, Watcho P, Matei H, Kamanyi A. Dracaena arborea alleviates ultra-structural spermatogenic alterations in streptozotocin-induced diabetic rats. BMC Complement Altern. Med. 2013;13:71.

55. Adelakun SA, Omotoso OD, Aniah JA. Modulating role of D-Ribose-L-cysteine on oxidative stress in streptozotocin induced diabetes on plasma lipoprotein, oxidative status, spermato-genesis and steroid-genesis in male wistar rats. Curr Res Diabetes Obes J. 2018;9(2):1-7
56. Ballester J, Munoz MC, Dominguez J, Sensat M, Rigaut T, Guinovart JJ, Rodrıguez-Gi JE. Insulin-dependent diabetes affects testicular function by FS Hand LH-linked mechanisms. J Androl. 2004;25:706-19.
57. Suthagar E, Soudamani S, Yuvaraj S, Ismail AK, Aruldhas MM and Balasubramanian K. Effects of streptozotocin (STZ)-induced diabetes and insulin replacement on rat ventral prostate. Biomed. Pharmacotherapy. 2009;6(3):43-50.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/53370