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Long Term Effects of Streptozotocin Induced Diabetes Mellitus on Hepatic, Nephrotic and Cardiac Physiology of Female Wistar Rats

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

Abstract

Background: Diabetes mellitus is a metabolic disorder which leads to complications that affect the heart, kidney, liver and other vital organs. Most reports on diabetes have focused on diabetes induction using male rat models in short term studies.

Objective: Here, we described the induction of diabetes in female rat and the complications of diabetes following long term hyperglycemia in female Wistar rats.

Method: Rats were assigned into two groups: control (n=10) and diabetic (n=10). The latter was administered a single (50mg/Kg body weight) intraperitoneal injection of streptozotocin. Bodyweight and blood glucose were assessed for 14 weeks, after which the animals were sacrificed and biochemical and morphology parameters of the liver, kidney and heart were determined.

Results: Diabetic rats showed continuous emaciation post STZ induction. The glycated haemoglobin, protein level, lipid profile, liver and kidney function markers were significantly different ($p<0.05$) from the control. Catalase, superoxide dismutase and glutathione-s-transferase activities were also significantly reduced in the heart, liver and kidney, and a concomitant increase in malondialdehyde concentration was observed. Focal tubular necrosis, pulmonary congestion and mild hyperplasia of kupffer cells were observed in the tissues and all tissues showed negative CD79a expression.

Conclusion: This study provides data that could be useful for modelling long-term diabetes mellitus studies in female experimental rat models.

Keywords: Oxidative stress, STZ-induced diabetes, Female Wistar rats, Diabetes complications

INTRODUCTION

Diabetes mellitus is a carbohydrate metabolism disorder that results in glucose aberration, leading to dysfunctions in the heart, kidney, liver, brain and other vital parts of the body (Brownlee, 2001). According to the WHO report in 2014, there were 422 million diabetic patients worldwide (Mekala and Bertoni, 2020). Its prevalence in Africa increased from 3.1% to 7.1% between 1980 and 2014 (Mekala and Bertoni, 2020). Diabetes mellitus is characterized by hyperglycemia and mortality due to acute or chronic complications (King, 2017). The former is mainly characterized by diabetic ketoacidosis while the latter is associated with the long-term effect of hyperglycemia on the vasculature, such as neuropathy, nephropathy, retinopathy, and cardiovascular disease (Bagheri, 2014). These complications can arise both in Type 1 and Type 2 diabetes (King, 2017).

Streptozotocin (STZ) is a chemical compound analogous to glucose that can destroy the pancreas' beta cells, thereby forestalling insulin synthesis inducing diabetes mellitus (Eleazu et al., 2013). STZ is a diabetogenic agent initially found as a broad-spectrum antibiotic from *Streptomyces acromogenes* and has been used since it was described first in 1963 (Rajab, 1993). This compound acts by basically impairing glucose metabolism and basement membrane alterations causing an alteration to the pancreatic cells impairing glucose homeostasis leading to Diabetes mellitus (Satav & Katyare, 2004). Despite the extensive research carried out using STZ as a model of diabetes induction, the long-term complications have not been explicitly studied extensively in female Wistar rats. We had earlier demonstrated the effect of long term diabetes on the reproductive function of female Wistar rats (Olawale et al., 2020). The current study provides some insight into how chronic complications of diabetes affect the liver, kidney and heart of STZ induced diabetic rats.

METHODOLOGY

Animals

Female Wistar rats (130-150g) were obtained from the animal house of the Department of Biochemistry, University of Ibadan, Nigeria and were used for the study. Rats were handled according to the guidelines by the Animal Care Unit Research Ethics Committee (ACUREC), University of Ibadan, Nigeria. The animals were placed in cages with 12-h dark/light cycles at an ambient temperature of 25 ± 2°C. Before treatment, they were acclimatized for two weeks and were fed with standard rat pellet and water *ad libitum*.

INDUCTION OF STZ

Diabetes was induced by a single 50 mg/kg intraperitoneal injection of STZ (Santa Cruz, USA). Briefly, rats were weighed and injected with STZ dissolved in a citrate buffer (0.1M, pH 4.5). After 72h, rats were anaesthetized; blood samples were collected about 2 mm from the tail's tip to determine the blood glucose using a clinical glucometer and commercially available test strips. Rats with plasma glucose levels more than 250 mg/dl were considered diabetic.

Animal Grouping

Twenty animals were equally randomized into two groups viz:

Control: control rats were fed with standard rat pellets

Diabetic: Diabetic rats were fed with standard rat pellets

Animals in the diabetic groups were administered with STZ. The weight and blood glucose of the rats were regularly assessed and recorded weekly.

Sacrifice and collection of organs

After 14 weeks, the rats were fasted overnight, anaesthetized using diethyl ether, and sacrificed. The whole blood sample was collected in plain tubes via ocular puncture. The blood samples were centrifuged at 4000 rpm for 15 minutes, and the supernatant collected and stored at 4°C to maintain enzyme activity for further analysis. The liver, kidney and heart were exercised, trimmed off fatty tissue, washed in 1.15% KCl solution and weighed. The animals' final body weights were also recorded while the relative organ weight was measured as the ratio of the organ weight to the bodyweight expressed in percentage.

Tissue preparation for Biochemical analysis and histological examination

About fifty percent of the organ was homogenized in four volumes of phosphate buffer (pH 7.4) using a Teflon homogenizer. The resulting homogenate was centrifuged at 10,000 rpm for 15 minutes in a cold centrifuge (4°C). The supernatant was collected and stored and used for biochemical analysis. The remaining portion of the organs was fixed in 10% formal saline for histological and immunohistochemistry studies.

Biochemical assays

Determination of physiological parameters

Serum glycated haemoglobin was determined enzymatically using test Kits (Clover A1c) (Hirokawa et al., 2005). Serum and tissue total protein (TP) levels were determined according to the method described by Lowry *et al*. (Randall and Lewis, 1951), using Bovine Serum Albumin as standard.

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were determined in the serum and liver homogenates using the method described by Mohun and Cook (Mohun and Cook, 1957) and by Reitman and Frankel, respectively (Reitman and Frankel, 1946). Alkaline phosphatase (ALP) activity was determined, as described by Eaton (Eaton, 1977). Serum bilirubin(bil) (direct and total bilirubin) was determined by the Colorimetric method described by Jendrassik and Gof (Lo and Wu, 1983).

Urea and creatinine levels were determined in serum and kidney homogenate according to the method of Fawcett and Scott (Fawcett and Scott, 1960); Jaffe reaction method (Slot, 1965) respectively.

Total cholesterol (CHOL) level was determined using enzymatic hydrolysis described by Roeschlau et al., 1974. Serum High-density lipoprotein (HDL)-cholesterol was determined by the method of Lopes-Virella et al., 1977.

Determination of oxidative stress biomarkers

Catalase (CAT) activity was determined according to the method of Claiborne (Claiborne, 1985).

Superoxide dismutase (SOD) activity was determined by the method of Mistra and Fridovich (Mistra and Fridovich, 1972).

Glutathione-S-transferase (GST) activity was determined according to the method described by Habig et al., 1974.

Lipid peroxidation was determined by measuring malondialdehyde (MDA) -an end product of lipid peroxidation as described by (Buege and Aust, 1978).

Histopathology

The organs were retrieved from formalin, blocks embedded in paraffin and sections cut at 5µ, which was then stained with H&E (Hematoxylin and Eosin) and mounted in Canada balsam. Microscopic examination of the sections was carried out under a light microscope (×400).

Immunohistochemistry

Liver, kidney and heart tissues were stained for CD79a (Igα) using rabbit anti-rat CD79a antibody (Novocastra kit). The principle is based on indirect detection, where an unlabelled primary antibody (dilution ratio 1:100) is recognized by a labeled secondary antibody upon binding to a specific antigen. The formation of antigen-antibody complex causes agglutination and the bands' intensity was quantified using Image J software.

Statistical analysis

Quantitative data were compared using a two-tailed student t-test for data analysis using Graph Pad Prism 5 (Graph Pad Inc. San Diego, USA). The significant difference was set at $p< 0.05$, and results reported as mean ± SD.

RESULTS

Anthropometric measurements

STZ administration led to a significant decrease in body weights compared to the control, which showed a significant increase in body weights (Figure I). The liver, kidney and heart showed a decrease in the Diabetic rats as compared with the control. There was a significant difference in the relative weights of the kidney of diabetic as compared with control (Table 1).

Table 1. Effect of STZ induced diabetic rats on the absolute and relative weights of organs

Parameter	Tissue	Control	Diabetic
Organ weight (g)	Heart	0.66±0.10	0.59±0.06
	Liver	6.21±0.95	5.61±0.83
	Kidney	1.03±0.10	1.08±0.10
%Relative organ weight	Heart	0.33±0.085	0.48±0.116
	Liver	3.1±0.74	4.60±1.5
	Kidney	0.51±0.10	0.88±0.20*

*Data are given as means ± S.D (n=5) *indicates significantly different as compared with the control (p<0.05)*

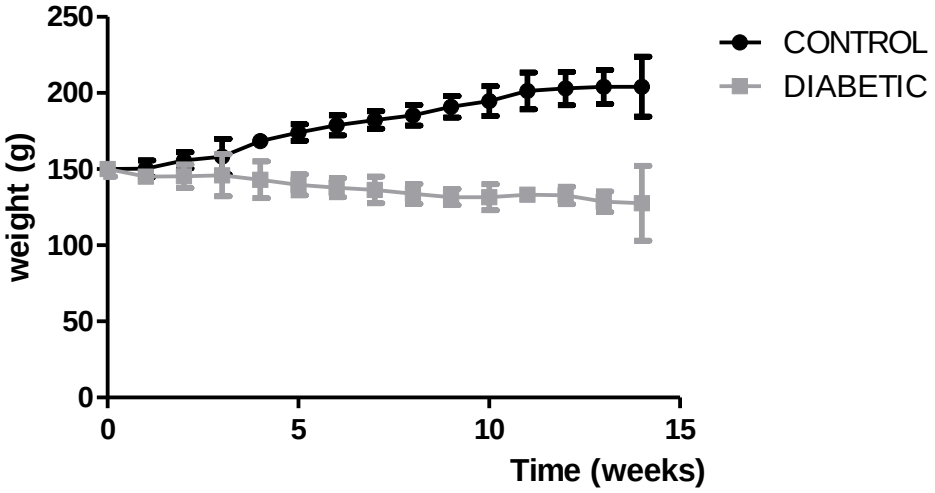

Fig.1: Weekly assessment of rat body weights of control and diabetic rats for 12 weeks. The results are given as mean ± S.D (n=5).

Diabetic markers, Protein estimation and Lipid profile bio-indicators

There was no significant difference between the initial blood glucose levels of the control and STZ treated groups before the induction of diabetes. However, there was a statistically significant increase in blood glucose of diabetic rats compared to the control following STZ induction (Figure 2). Diabetic rats also had a marked increase in glycated haemoglobin (HbA1c) and total cholesterol compared with the control (Table 2). Also observed is a significant decrease in serum protein and HDL-CHOL of diabetic rats compared with the control.

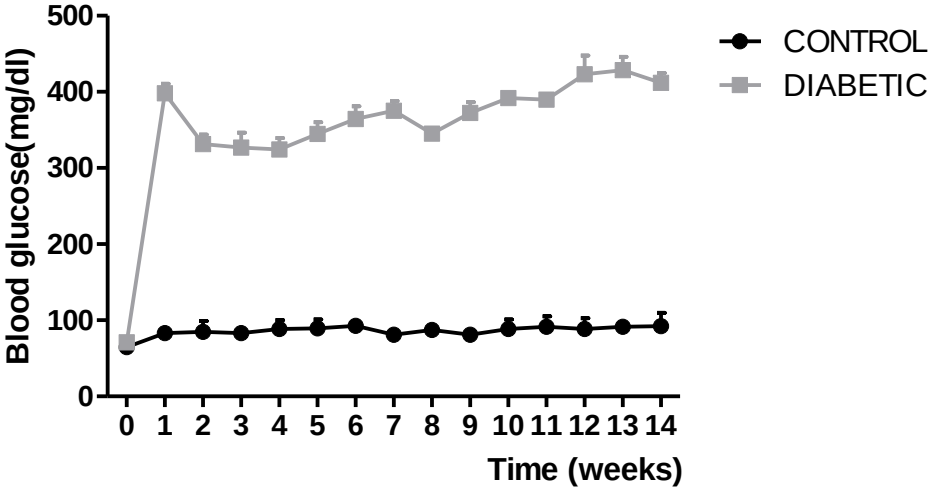

Fig. 2. Weekly evaluation of blood glucose in control and diabetic rats for 14 weeks. The results are given as mean ± S.D (n=5).

Liver function indicators and Kidney biomarkers

There was a significant increase in the level of AST, ALT, ALP enzymes present in the serum compared to the control, which was also accompanied by a significant decrease in ALT level, AST enzyme in the liver tissues of diabetic rats (Table 2).

Table 2: Mean biochemical indices, activity of transaminases and kidney biomarkers in serum and tissues of diabetic rats

Parameter	Tissue	Control	Diabetic
ALT(µ/l)	Serum	27.08±1.79	53.06±4.15*
	Liver		
		89.32±4.85	73.44±7.00*
	Kidney		
		86.70±4.39	71.96±4.00*
AST(µ/l)	Serum	28.32±3.54	51.68±3.11*
	Liver		
		92.70±3.95	74.61±4.34*
	Kidney		
		25.80±3.89	17.98±3.57
ALP(µ/l)	Serum	113.42±8.35	337.78±9.60*
Creatinine(mg/dl)	Serum	0.86±0.31	1.52±0.33*
	Kidney		
		3.82±0.23	2.38±0.44*
Urea (mg/dl)	Serum	15.51±0.80	25.4±3.70*
	Kidney		
		34.34±4.55	14.24±3.19*
Total bil (mg/dl)	Serum	3.14±0.49	2.88±0.44
Direct bil (mg/dl)	Serum	0.728±0.12	1.98±0.57*
HbA1c (%)	Serum	4.62±0.63	8.08±0.61*
T. CHOL (mg/dl)	Serum	4.13±0.10	4.96±0.19*
HDL (mg/dl)	Serum	2.3±0.62	1.024±0.80
TP(mg/g)	Serum	3.26±0.15	1.12±0.19*

*Data are expressed mean±S.D (n=5) *indicates significantly different as compared with the control (p<0.05)*

There was also a marked significant increase in the conjugated bilirubin level despite the higher total bilirubin level in the diabetic group. There was a significant increase in the serum level of urea and creatinine in the diabetic group and a significant concomitant decrease in the kidney tissue level in the diabetic group. Also observed was a significant decrease in the kidney ALT level of the diabetic group.

Oxidative stress biomarkers

There was a significant decrease in catalase enzyme expression in the liver, heart and kidney tissue of the diabetic group compared to the control (Table III). The SOD enzyme was also less expressed in the diabetic liver and kidney. The expression of GST enzyme showed a significant decrease in the heart and liver of the diabetic group as compared to control rats. The MDA concentration was shown to be significantly increased in all tissues in the diabetic group.

Table 3. Antioxidant enzyme profile and MDA concentration in tissues of experimental groups

Parameters	Tissue	Control	Diabetic
CAT(U/mg protein)	Heart	46.82±5.41	14.92±5.10*
	Liver	52.22±5.15	15.02±3.49*
	Kidney	37.96±5.15	15.72±4.71*
SOD(U/mg protein)	Heart	27.14±3.45	22.52±2.71
	Liver	31.48±3.95	23.76±3.05*
	Kidney	22.82±3.45	16.50±2.59*
GST(µmol/min/mg protein)	Heart	17.75±3.23	12.32±2.76*
	Liver	19.74±4.30	12.56±2.00*
	Kidney	9.70±1.89	5.90±1.89
MDA(µmol/mg protein)	Heart	2.02±0.36	5.94±0.65*
	Liver	1.98±0.40	5.34±0.64*
	Kidney	2.48±0.36	5.34±0.32*

*Results are given as mean ± S.D (n=5) *indicates significantly different as compared with the control (p<0.05)*

Histological Examinations

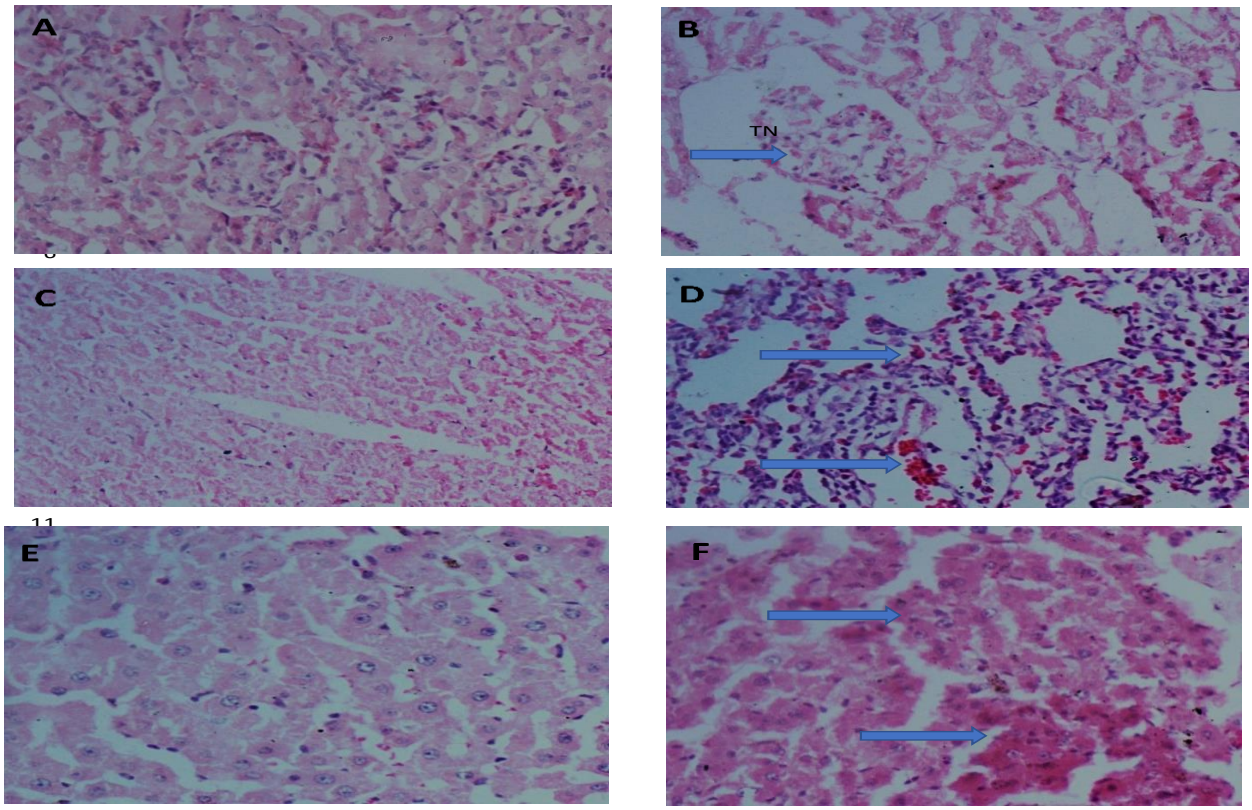

Figure 3. Photomicrographs of hematoxylin, and eosin-stained kidney sections (A-B), heart sections (C-D), liver sections (E-F) from control and diabetic rats. (A) Control rats kidney shows the typical architecture of renal corpuscles and renal tubules. (B) Diabetic rats kidney tissue shows a sparse population of renal tubules; there are also signs of focal tubular necrosis (TN). (C) Control heart follows standard architecture made up of three layers endocardium, myocardium and epicardium. (D) Diabetic heart tissues show increased myocardial layer and pulmonary congestion. (E) Control liver tissues show normal ultrastructure. (F) Shows hepatic plates were closely packed and there is mild kupffer cell hyperplasia

Immunohistochemistry

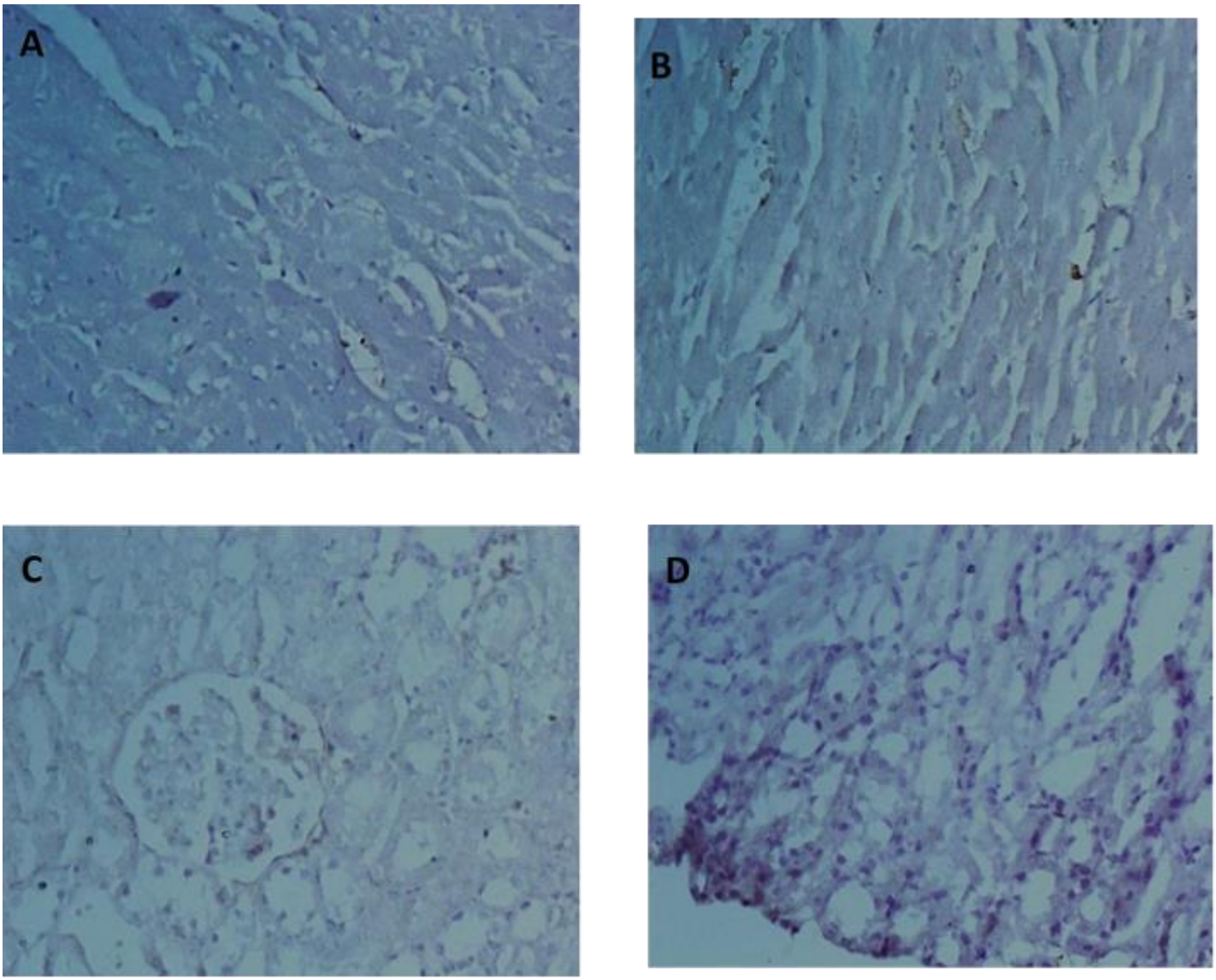

Figure 4: Immunohistochemistry staining of CD 79 in (A) control heart (B) diabetic heart (C) control kidney (D) diabetic kidney after 14 weeks post STZ induction in female Wistar rat. Immunoreactivity quantification of CD79 in all the tissues(A-D) shows no CD 79 expression.

DISCUSSION

Diabetes mellitus is a metabolic disorder associated with mortalities due to the consequent effects of hyperglycemia on the kidney, liver, heart and other vital organs leading to complications such as diabetic nephropathy, cardiomyopathy and varying liver abnormalities (Adiga and Malawadi, 2016; Brownlee, 2001).

In our experiment, a progressive decrease in body weight was observed weekly, similar to that observed by Wei *et al*., (2003). This is due to increased muscle wasting and loss of tissue protein which corroborates the findings by Cheng *et al*., (2013). This general loss in mass is observed in patients with untreated type 1 and type 2 diabetes mellitus which experience diabetic ketoacidosis and hyperosmolar hyperglycemia (Umpierrez, 2018).

It was shown that the blood glucose increased significantly three days post-STZ induction and increased successively over a slight range till the end of the experiment. The sudden increase was observed since a single high dose of STZ caused the destruction of the β-cells of the pancreas (Szkudelski, 2001).

Glycated haemoglobin, which is an abnormal haemoglobin found in diabetic patients, was significantly higher than the control and had a significant value of about 8.08% which is higher than the benchmark for non-diabetic patients (Weykamp *et al*., 2008). An elevated glycated haemoglobin level in the tissues suggests that a higher glucose level causes oxidative protein glycation (Ganesan, 2013; Seedevi *et al*., 2020).

STZ induced diabetes lead to a significant increase in serum ALT, AST levels and a concomitant decrease in the expression of these enzymes in the liver and kidney tissues indicating damage to the hepatic and renal tissues (Concepciónnavarro et al., 1992). A significant increase in serum urea and creatinine is indicative of kidney damage in diabetic rats. This damage can be linked to hyperglycemia-induced oxidative stress caused by increased ROS, which can assault various target organs, including the kidney (Brownlee, 2001). Increased ALP level portrays hepato, nephro and cardiotoxicity (Erukainure *et al*., 2019) indicating damage to the liver, kidney and heart.

Diabetes mellitus is associated with various metabolic disorders, such as lipid metabolism (Seedevi *et al*., 2020). Our results show an increased level of total cholesterol and decreased HDL level leading to secondary complications of diabetes (Arvind and Pradeepa, 2002). Increased cholesterol observed suggest hyperlipidemia which is a risk factor in the progression of diabetic nephropathies (Wen et al., 2002). Hypercholesterolemia triggers pro-inflammatory events, and it is a risk factor for coronary heart disease (Li et al., 2014). The increase in serum lipids in diabetic rats indicates rapid mobilization of free fatty acids from the adipose tissue (Geethan and Prince, 2008) causing weight loss as observed in our experiment which is a hallmark for diabetic ketoacidosis (Umpierrez, 2018).

The increased level of conjugated bilirubin observed is indicative of biliary obstruction, which consequently affects lipid metabolism. This might be a contributing factor to the increased serum lipids level in diabetic rats. Individuals with diabetes are at a significantly greater risk of developing both micro and macrovascular diseases and have a cardiac mortality equivalent to that in non-diabetic patients with confirmed heart disease (Badole and Jangam, 2015). The diabetic rats in our experiment displayed dyslipidemia, microalbuminuria and cardiomyopathies, which are standard features observed in human diabetes, contributing to a high prevalence of cardiovascular diseases (Wei *et al*., 2003).

Oxidative stress has been associated with various pathological conditions in humans, including diabetes. The oxidative stress markers in the study showed a marked reduction in the level of antioxidant enzymes in all the tissues. Catalase appears to be the most affected. A marked increase in MDA concentration corroborates these results. These findings support the previous studies that oxidative stress contributes to the progression and complications of diabetes (Rashid *et al*., 2019).

The tissue degeneration observed in the diabetic rat indicates damage to the liver, kidney and heart (Figure III). Furthermore, diabetic patients experience severe hyperglycemic complications such as myocardial infarction, diabetic nephropathy, and liver inflammation as observed from the histological examination of the heart, kidney and liver tissues of STZ treated diabetic rats.

CD79 is a covalent disulfide heterodimer composed of two subunits: CD79a (Igα) and CD79b (Igβ) (Polson et al., 2007). It is a signalling component of B-cell Receptor (BCR) of the B-lymphocyte Cross-linking of BCR triggers apoptosis in some cells (Polson et al., 2007). Hyperglycemia has been linked to cell apoptosis and necrosis (Schmatz et al., 2012). However, negative expression of CD79a protein in diabetic rats' heart and kidney (Figure IV) indicate that a BCR receptor-mediated apoptosis pathway is not a possible mechanism of the cell damage in these tissues and b-cell recruitment to the hepatic, cardiac and nephrotic cell does not occur in STZ induced diabetic female Wistar rats.

CONCLUSION

The result has thus far demonstrated that prolonged hyperglycemia in STZ induced diabetes in female Wistar rats leads to pathological conditions in the liver, heart and kidney which is similar to that observed in human diabetic subjects with severe diabetes mellitus. It was shown that oxidative stress is implicated in the cellular damage observed in the liver, kidney and heart of diabetic patients, and these occur via a non-b-cell-dependent apoptotic pathway and cellular necrosis.

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