

Biochemical, Hormonal and Histological Changes in Prostate of Wistar Rats Following Long Term Streptozotocin-induced Diabetes Mellitus

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Summary: Diabetes mellitus (DM) is characterized by hyperglycemia and endocrine disorder. Diabetes mellitus is known to promote male infertility by affecting sperm quality through altered steroidogenesis. The role of prostatic fluid in maintenance of sperm quality have been established. However, the effect of DM on prostate health is poorly understood. This study was designed to investigate the biochemical, hormonal and histological changes in prostate of male Wistar rats after 3- and 5-months DM. Twenty-six adult male Wistar rats were assigned into three groups. Group I (non-diabetic rats) served as control (n=10), out of which five rats (n=5) were sacrificed as control for 3-month study and the remaining animals (n=5) as control for 5-month study. Group II served as 3-month DM (n=8) and group III served as 5-month DM (n=8). Diabetes mellitus was induced by administration of a single dose of streptozotocin (STZ) (35 mg/kg i.p.). Rats were sacrificed at 3- and 5- months after DM. Biochemical indices in serum and prostate, histological and immunohistochemical studies of the prostate were evaluated. Results indicated that the weight of prostate of 3- and 5-months DM rats significantly ($p<0.05$) decreased by 33% and 59%, respectively. Fasting blood glucose, plasma glycated haemoglobin and number of micronucleated polychromatic erythrocytes in the bone marrow significantly ($p<0.05$) increased in 3- and 5-months DM. Activities of serum alanine and aspartate aminotransferases significantly ($p<0.05$) increased in 3 months DM with concomitant increase in serum total bilirubin and urea in both models. The activities of total acid phosphatase in 3- and 5-months DM decreased by 34% and 76%, respectively while prostatic acid phosphatase decreased by 48% in 5-months DM. Prostatic zinc and bicarbonate increased in 3-months DM by 1.7 and 1.9 folds, and in 5-months DM by 5.8 and 1.7 folds, respectively. The levels of serum luteinizing and follicle stimulating hormones and testosterone decreased in both models. Prostatic lipid peroxidation increased while activities of antioxidative enzymes decreased in both models. Histology revealed hyperplasia, intra-luminal budding of epithelia, mild expressions of Bcl₂ and Ki67 in 3- and 5-months DM. Overall, prostate health was compromised and may increase infertility in diabetic animals.

Keywords: Diabetes mellitus; Prostate, Hormones; Antioxidant enzymes; Oxidative stress

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INTRODUCTION

Diabetes mellitus (DM), characterized by hyperglycemia, is a chronic metabolic and endocrine disorder that is associated with defects in insulin secretion and/or sensitivity. Hyperglycemia, a hallmark of DM promotes oxidative stress in the presence of mitochondrial glucose oxidation (Chang and Chuang, 2010) which later serve as one of the factors responsible for micro- and macro-vascular complications (Giacco and Brownlee, 2010). Vignera *et al.*, (2012) reported that 35% of DM patients are infertile and, the infertility has been linked to reduce sperm quality. Loss of sperm function in DM is often associated with oxidative stress (Gomez *et al.*, 1996). In addition to poor sperm quality, derangements in the endocrine control of spermatogenesis in DM have been reported (Rato *et al.*, 2013; Alves *et al.*, 2013). For example, abnormal synthesis of testosterone due

to structural and molecular changes in the Leydig cells has been linked to male infertility in DM (Dinulovic *et al.*, 1990). Also, pituitary-gonadal system also interferes in these endocrine disorders found in DM (Bhasin *et al.*, 2007). It is now suggested that prostate health may contribute to male diabetic infertility since the volume of prostatic secretions may reduce during prostate gland pathology (Weidner *et al.*, 1999). Also, La Vignera *et al.*, (2013) reported that semen viscosity may increase in infertile patients with prostate gland pathology and, the increased viscosity may adversely affect semen parameters, especially sperm motility (Elia *et al.*, 2009). Several studies have reported direct relationship between diabetes and prostate pathologies (Kasper and Giovannucci, 2006; Tseng 2011 and Bansal *et al.*, 2013), which may be a consequence of metabolic aberrations and alterations in sex hormone levels in diabetic patients (Bonovas *et al.*, 2004). Moreover, pathologies of prostate, triggered by

decreased insulin secretion or resistance to insulin action (Meyer *et al.*, 2000), may lead to decrease in prostatic secretions (Marconi *et al.*, 2009). These factors, therefore play significant roles in prostate functions, from enzymatic activity to sperm motility. Hence, the status of prostate with respect to biochemical, anatomical and hormonal changes during DM needs to be properly understood in order to reduce the menace of infertility in male diabetic subjects. Thus, this study was designed to investigate biochemical, hormonal and histological changes in prostate of male Wistar rats after long term streptozotocin-induced diabetes mellitus.

MATERIALS AND METHODS

Chemicals and reagents. Streptozotocin, glutathione, hydrogen peroxide, 5,5'-dithio-bis-2-nitrobenzoic acid (DNTB) and epinephrine were purchased from Sigma Chemical Co., Saint Louis, MO, USA. Trichloroacetic acid and Thiobarbituric acid were purchased from British Drug House (BDH) Chemical Ltd., Poole, UK. Other chemicals and reagents were of analytical grade and purest quality available.

Animals. Adult male Wistar rats were purchased from the animal house of the Department of Veterinary Physiology, University of Ibadan, Nigeria for the study. They were kept in ventilated cages at room temperature (25-30°C) and maintained on normal laboratory chow (Ladokun Feeds, Ibadan, Nigeria) and water *ad libitum*. Animals were allowed two weeks to acclimatize before the commencement of study. All experimental procedures were carried out in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals. The study was approved by the University of Ibadan Animal Ethics Committee.

Study design. Twenty-six animals (Sixteen diabetic and ten non-diabetic) were divided into three groups. Group I (non-diabetic rats) served as control (n=10), out of which five rats (n=5) were sacrificed as control for 3-month study and the remaining animals (n=5) as control for 5-month study. Group II served as 3-month DM (n=8) and group III served as 5-month DM (n=8). Diabetes mellitus was induced by the administration of a single dose of streptozotocin (STZ) (35 mg/kg i.p.) dissolved in 0.1M citrate buffer (pH 4.0) (Adaramoye *et al.*, 2012). After 72 h of STZ administration, blood glucose levels of the rats were determined using Accu-Check Glucometer (Roche) with compatible glucometer strips. Animals with blood glucose >250 mg/dL were considered to be diabetic and selected for the study. Animals in each group were assessed fortnightly for signs of weakness, cataract, weight loss, hair loss, coma and death.

Sample Collection. At the end of the experiments (3 and 5 months), animals (n=5) each in groups II and III survived) and were fasted overnight, and euthanized

under light ether anesthesia. Part of blood samples were collected into EDTA bottles and, used for glycated haemoglobin (HbA1c) assay while remaining blood samples were collected in plain tubes and were allowed to clot. The clotted blood was centrifuged at 3000 g for 10 minutes to obtain serum for the determination of AST, ALT, Bilirubin, LH, FSH and Testosterone.

Preparation of prostatic tissues. The prostates were excised and washed in ice-cold 1.15% KCl solution, dried and weighed. Sections of the prostate were fixed in formalin for histology and immunohistochemical analyses. The remaining portions were homogenized in 6 volumes of 50 mM phosphate buffer, pH 7.4 and centrifuged at 10,000 x g for 15 min to obtain post-mitochondrial supernatant fraction (PMF). The PMF collected was stored at -80°C for biochemical analysis (SOD, CAT, GSH, GPx, LPO, ACP, ALP, Zn and bicarbonate ions).

Biochemical assays

Protein determination. Serum and prostatic protein levels were determined according to the method of Lowry *et al.*, (1951) using bovine serum albumin as standard.

Glycated haemoglobin determination. The level of glycated haemoglobin in the whole blood was estimated with CLOVER A1C® Self test kit using auto analyser.

Determination of acid and alkaline phosphatase activities. The activity of prostatic acid phosphatase was determined by the method of Fishman and Lerner (1953). Alkaline phosphatase (ALP) activity was measured spectrophotometrically by monitoring the concentration of phenol formed when ALP reacts with disodium phenyl phosphate at 680 nm as described by Williamson, (1972).

Alanine and aspartate aminotransferases determination. Serum alanine aminotransferase (ALT) and aspartate aminotransferases (AST) activities were determined using a combination of the methods of Mohun and Cook, (1957) and Reitman and Frankel, (1957).

Creatinine and urea determination. Serum creatinine and urea levels were estimated by the methods of Jaffe, (1886) and Talke and Schubert, (1965) respectively.

Micronucleus assay. Bone marrow fluids containing cells were harvested, prepared and stained with Giemsa stain. The number of micronucleated polychromatic erythrocytes (mnPCEs) per 1000 polychromatic erythrocytes (PCEs) was counted under light microscope. The ratio of mnPCE to normochromatic erythrocytes (PCE/NCE) was calculated. While normal cells appeared pink,

damaged cells appeared blue, with the micronucleus appearing as a black spot in the cells.

DNA fragmentation by diphenylamine (DPA) assay. The colorimetric determination of fragmented DNA was done according to the method described by Wu et al., (2005) with slight modification. The prostate samples were homogenized in Tris-EDTA buffer and centrifuged at 27000 x g for 10 minutes, to separate the intact DNA (pellet) from the fragmented DNA (supernatant). Both the pellet and supernatant were treated with freshly prepared diphenylamine reagent for colour development. Incubation was carried out at 37°C for 20-24 hours. The absorbance was read spectrophotometrically at 620 nm. The percentage fragmented DNA was calculated using the formula:

$$\% \text{ fragmented DNA} = \frac{\text{Absorbance of supernatant}}{\text{Absorbance of pellet} + \text{Absorbance of supernatant}} \times 100$$

Determination of zinc and selenium levels. Prostatic Zn and Se levels were determined by direct method using atomic absorption spectroscopy (AAS), Shimadzu model AA-580 fitted with a Shimadzu PR-5 graphic printer following the manufacturer's analytical techniques at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. The determination of Selenium concentration was done by digesting the sample with a mixture of nitric and perchloric acid. After hydride generation and using a sodium borohydride method, the selenium concentration was determined (AAS Model AA-580). To monitor the reproducibility and accuracy of the analytical techniques, reagent blanks and known samples were interspersed with the test samples.

Antioxidant assays. Prostatic superoxide dismutase (SOD) activity was assayed by the method of McCord and Fridovich, (1969). Catalase (CAT) activity was determined spectrophotometrically by measuring the rate of decomposition of hydrogen peroxide at 240 nm as described by Aebi, (1974). Reduced glutathione (a non-enzymatic antioxidant) level in the prostate was measured by the method of Beutler *et al.*, (1963), which is based on the development of a relatively stable (yellow) colour when 5',5'-dithiobis-(2-nitrobenzoic acid) (Ellman's reagent) is added to sulfhydryl compounds. The chromophoric product resulting from the reaction of Ellman's reagent with the reduced glutathione (2-nitro-5-thiobenzoic acid) possesses a molar absorption at 412 nm that is proportional to the level of reduced glutathione in the test sample. Glutathione peroxidase (GPx) activity was determined by the method of Rotruck *et al.*, (1973) while Glutathione-S-transferase (GST) activity was measured according to Habig *et al.*, (1974), the method is based on the fact that all known GST demonstrates a relatively high activity with 1-chloro-2,4-dinitrobenzene as the second substrate. When this substance is conjugated with reduced glutathione, its

absorption maximum shifts to a longer wavelength of 340 nm and the increase in absorption at this wavelength provides a direct measurement of enzymatic reaction. Prostatic lipid peroxidation level was assayed by the reaction between 2-thiobarbituric acid (TBA) and malondialdehyde (MDA), an end product of lipid peroxidases described by Buege and Aust, (1978). On heating at acidic pH, the product is a pink chromophore which absorbs maximally at 532 nm and it is extractable into organic solvents such as butanol. MDA is often used to calibrate this test and results are expressed as the amount of free MDA produced.

Hormonal assays Serum testosterone was assayed by the enzyme-linked immunoabsorbent assay (ELISA) as described by Tietz (1995) using Serozyme I Sero (Diagnostics, Freiburg, Germany). The testosterone concentration was obtained by correlating the absorbance of the test sample at 550 nm with the corresponding absorbance on the standard curve. The FSH and LH concentrations were determined based on a solid-phase enzyme-linked immunoabsorbent assay as described by Uotila *et al.*, (1981).

Immuno-histochemical assay

The immunochemical staining of prostate for expression of Bcl₂ and Ki67 were done using novocastra™ kit according to the method of Chakravarthi *et al.*, (2010). The principle is based on the binding of a primary antibody (dilution of 1:100) to a specific antigen. The antibody-antigen complex formed is incubated with a secondary, enzyme-conjugated antibody. In the presence of substrate and chromogen, the enzyme acts on the substrate to generate colored deposits at the sites of antibody-antigen binding which was observed under a binocular microscope. Cells with specific blue color in the cytoplasm, cell membrane or nuclei, depending on the antigenic sites were considered positive, and compared with external controls. Antigen retrieval was performed on the sections by heating in a citric acid buffer (pH 6.0) at 100°C for 15 minutes. Bands intensities were quantified using ImageJ software.

Histology.

The prostates fixed in 10% formalin were dehydrated in 95% ethanol and then cleared in xylene before embedded in paraffin. Micro sections (4 μm) were prepared and stained with haematoxylin and eosin (H&E) dye, and were examined under a light microscope by a Histopathologist who was ignorant of the treatment groups.

Statistical analysis.

Data were expressed as the Mean ± S.D. The values were analyzed using one-way ANOVA followed by the post-hoc Duncan's multiple range test for analysis

of biochemical data using SPSS (10.0). Values were considered statistically significant at $p < 0.05$.

RESULTS

Effects of DM on the levels of blood glucose, glycated hemoglobin and weight of prostate

Diabetes mellitus caused a significant ($p < 0.05$) decrease in weight of prostate of 3- and 5-months diabetic rats by 33% and 59%, respectively when compared to the control. Also, the body weight-gain of 3- and 5-months DM rats decreased by 41% and 97%, respectively relative to controls (Table 1). In addition, DM also significantly ($p < 0.05$) increased fasting blood glucose and glycated haemoglobin at both models when compared to controls (Table 2).

Effects of DM on serum biochemical parameters

Diabetes mellitus caused a significant ($p < 0.05$) increase in serum urea and creatinine at 3 and 5 months model by 56%, 57% and 46%, 45%, respectively (Table 2). In addition, the levels of total bilirubin decreased by 45% and 31%, respectively, in 3 and 5-months diabetic rats when compared to controls (Table 2). Furthermore, the levels of serum LH, FSH and

testosterone were significantly ($p > 0.05$) decreased in 3-months DM by 1.9, 2.3 and 3.1 folds, and in 5-months DM by 1.6, 2.1 and 4.7 folds, respectively (Table 3). Also, ALT and AST activities significantly ($p < 0.05$) increased in 3 months DM relative to controls. However, there were no significant differences ($p > 0.05$) in ALT and AST activities in 5 months diabetic rats relative to controls (Figure 1).

Effects of DM on the activities of prostate phosphatases, levels of prostatic zinc, selenium and bicarbonate of rats

The activities of total acid phosphatase (ACP) in the prostate of STZ diabetic rats decreased ($p < 0.05$) significantly at 3- and 5-months models when compared to control, with percentage decreases of 56% and 71%, respectively (Figure 2). Also, there was a significant decrease (48%) in the activities of prostatic ACP in 5 months diabetic rats when compared to the control. In addition, the activity of alkaline phosphatase (ALP) in the prostate of 3 months diabetic rats significantly ($p < 0.05$) increased by 28% when compared to the control. In contrast, in 5 months

Table 1: Changes in body weight, organ and relative weight of organ in 3 months and 5 months diabetic rats' model.

Group	3 Months model					5 Months Model				
	Body weight (g)			Prostate		Body weight (g)			Prostate	
	Initial	Final	Weight gain	Weight (g)	R.Wt (%) ¹	Initial	Final	Weight gain	Weight (g)	R.Wt (%) ¹
Control	252.6±9.51	298.6±5.96	50.2±6.84	0.45±0.06	0.15±0.01	248.9±8.56	295.4±8.57	46.46±11.16	0.39±0.03	0.13±0.01
Diabetic	188.9±5.64	222.6±7.69	29.4±1.80*	0.30±0.04*	0.14±0.03	181.9±1.67	183.3±4.94	1.40±0.65* ^a	0.16±0.01* ^a	0.09±0.01*

Values are expressed as Mean ± SD (n= 5). *Significantly different when compared to control ($p < 0.05$). ^a Significantly different when compared to 3 months ($p < 0.05$). R. Wt¹= Relative prostate weight as percentage of body weight

Table 2: Changes in serum biochemical indices in 3 and 5 months diabetic rats' model

Parameters	3 - months model		5 - months model	
	Control	Diabetic	Control	Diabetic
Glycated Haemoglobin (%)	3.50±0.02	7.00±0.52*	3.60±0.1	9.3±0.03*
Blood glucose (mg/dL)	131.60±2.90	496.0±14.77*	93.60±3.50	536.70±18.20*
Urea (mg/dL)	77.41±1.14	120.67±2.28*	50.51±2.18	73.88±1.25*
Creatinine (mg/dL)	5.41±0.55	8.57±1.19*	4.10±0.01	7.49±0.01
Protein (mg/g)	22.64±1.04	24.76±0.38	35.97±1.93	28.92±0.32
Bilirubin (Total) (mg/dL)	1.10±0.03	1.99±0.11*	1.03±0.08	1.49±0.16*
Bilirubin (Direct) (mg/dL)	0.78±0.07	1.72±0.13*	0.53±0.17	1.22±0.31
Bilirubin (Indirect) (mg/dL)	0.32±0.01	0.29±0.05	0.50±0.14	0.67±0.09
Bicarbonate (µg/dL)	1031.67±23.20	1920.00±16.10*	1225.00±35.00	2135.00±31.00*

Values are expressed as Mean ± SD (n= 5) * Significant different when compared to control ($p < 0.05$).

Table 3: Changes in serum hormones, zinc and antioxidative enzymes in 3 and 5 months diabetic rats' model

Parameters	3 - months model		5 - months model	
	Control	Diabetic	Control	Diabetic
LH (IU/L)	15.60±1.76	8.03±0.92*	7.00±1.03	4.30±0.70*
FSH (IU/L)	12.00±2.23	5.20±1.16*	4.50±0.89	2.07±0.50*
Testosterone (IU/L)	1.97±0.36	0.63±0.05*	1.64±0.03	0.35±0.05*
GSH (mg/g)	39.53±5.12	21.04±3.46*	37.71±0.46	15.42±0.38*
GPx (mg/g)	174.50±8.16	93.20±7.04*	219.63±7.34	103.28±5.34*
SOD (U/mg protein)	0.16±0.01	0.07±0.01*	0.20±0.01	0.03±0.004*
CAT (U/mg protein)	24.65±1.27	15.86±0.15*	159.60±5.76	101.20±9.19*
Zn (µg/dL)	1.00±0.03	1.67±0.67*	0.04±0.02	0.23±0.01*
Se (µg/dL)	49.21±6.83	48.68±7.57	23.46±2.20	31.91±0.76

Values are expressed as Mean ± SD (n= 5). * Significant different when compared to Control ($p < 0.05$).

DM, significant decrease ($p < 0.05$) in ALP activity was observed (Figure 3). There were significant increases ($p < 0.05$) in the levels of zinc and bicarbonate in prostate of 3- and 5-months DM rats while insignificant differences ($p > 0.05$) was observed in the selenium level (Tables 2 and 3).

Effects of DM on antioxidant indices, number of micronuclei and DNA fragmentation in rats

The level of GSH and activity of GPx in the prostate of STZ diabetic rats in both 3- and 5- months DM significantly ($p < 0.05$) decreased relative to controls (Table 3). Also, the activities of prostatic SOD, CAT and GST significantly ($p < 0.05$) decreased in the 3- and 5- months DM models (Table 3 and figure 4). In addition, DM caused significant ($p < 0.05$) increase in the prostatic LPO in both 3- and 5-months diabetic model were also observed when compared to the control. Specifically, the prostatic LPO increased by 1.2 and 1.4 folds in 3- and 5-months DM models, respectively (Figure 5). In figure 6, the number of micronucleated polychromatic erythrocytes (mnPCEs)

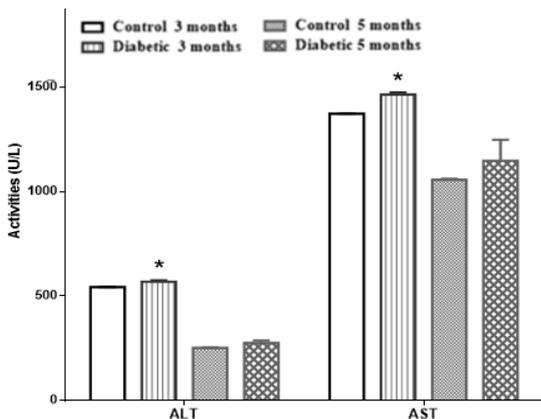


Figure 1. The activities of serum Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) in streptozotocin (STZ) diabetic rats. *Significantly different when compare with control

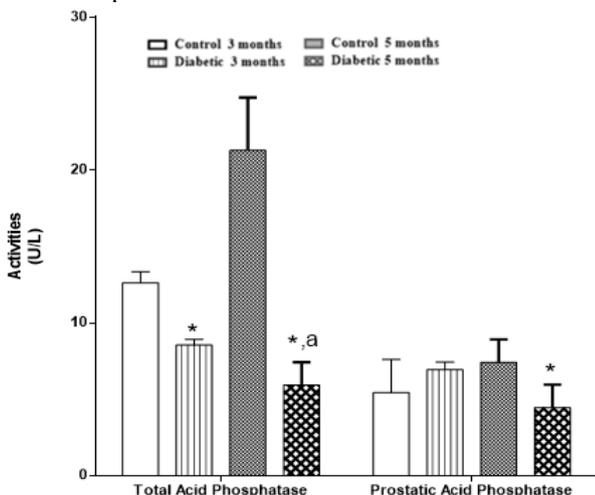


Figure 2. The activities of Acid Phosphatase (ACP) in prostate of streptozotocin (STZ) diabetic rats. *Significantly different when compare with control, ^aSignificantly different when compare with 3 months group.

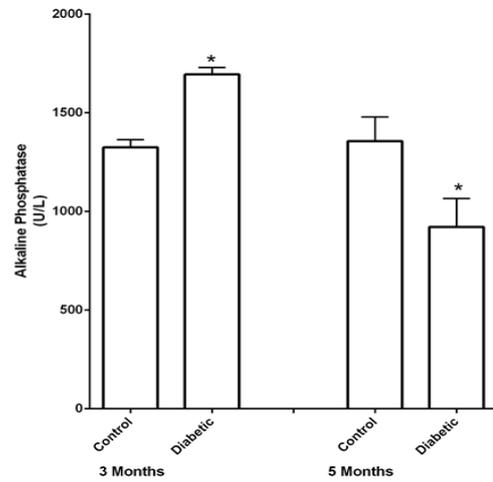


Figure 3. The activities of Alkaline Phosphatase (ALP) in prostate of streptozotocin (STZ) diabetic rats. *Significantly different when compare with control.

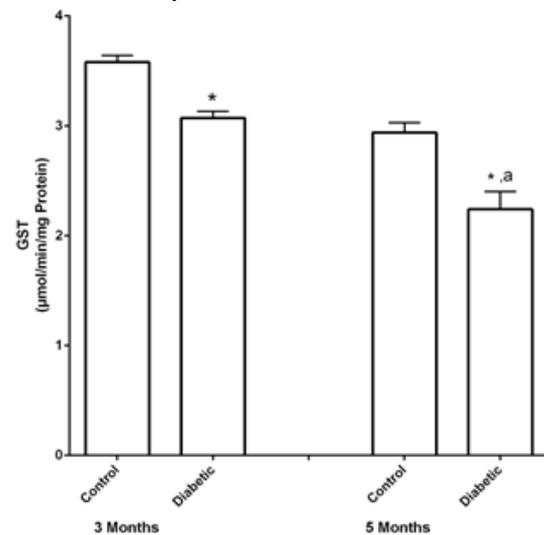


Figure 4. The activities of Glutathione-S-Transferase (GST) in prostate of streptozotocin (STZ) diabetic rats. *Significantly different when compare with control, ^aSignificantly different when compare with 3 months group.

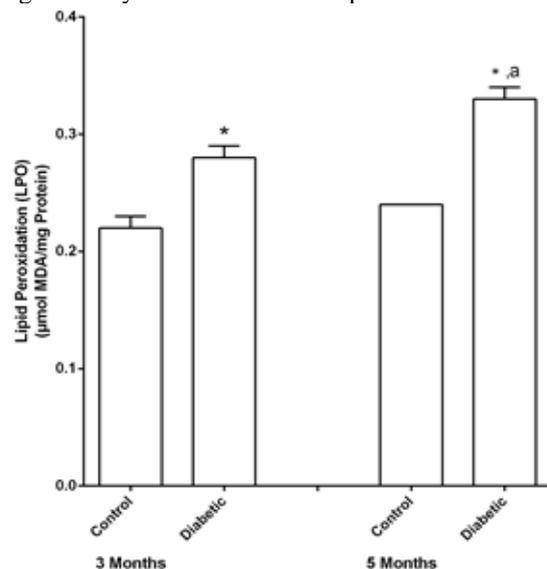


Figure 5. The level of prostatic lipid peroxidation in streptozotocin (STZ) diabetic rats. *Significantly different when compare with control, ^aSignificantly different when compare with 3 months group.

Effects of DM on antioxidant indices, number of micronuclei and DNA fragmentation in rats

The level of GSH and activity of GPx in the prostate of STZ diabetic rats in both 3- and 5- months DM significantly ($p < 0.05$) decreased relative to controls (Table 3). Also, the activities of prostatic SOD, CAT and GST significantly ($p < 0.05$) decreased in the 3- and 5- months DM models (Table 3 and figure 4). In addition, DM caused significant ($p < 0.05$) increase in the prostatic LPO in both 3- and 5-months diabetic model were also observed when compared to the control. Specifically, the prostatic LPO increased by 1.2 and 1.4 folds in 3- and 5-months DM models, respectively (Figure 5). In figure 6, the number of micronucleated polychromatic erythrocytes (mnpces) in bone marrow of diabetic rats increased by 112% and 159%, in 3- and 5-months DM respectively. There were no significant differences ($p > 0.05$) in the percentage of fragmented DNA in 3- and 5-months DM models relative to the control (Figure 7).

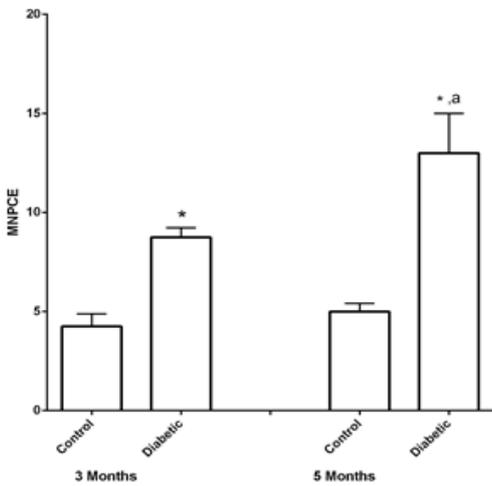


Figure 6. The frequency of Micronucleated Polychromatic Erythrocyte (MNPCE) in the bone marrow of Streptozotocin (STZ) diabetic rats. *Significantly different when compare with control, ^aSignificantly different when compare with 3 months group.

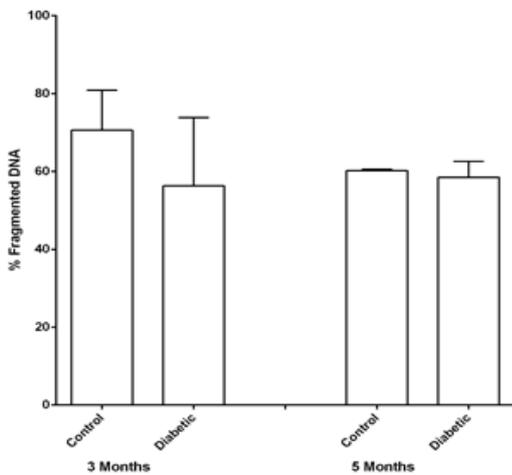


Figure 7. The Percentage of Fragmented DNA in the prostate of Streptozotocin (STZ) diabetic rats.

Effects of DM on the histology of prostate, expressions of Bcl₂ and Ki67

Histopathological examinations of prostate revealed distortion, hyperplasia with intraluminal budding of epithelia cells in 3- and 5-months diabetic rats (Figures 8B and C) relative to control (Figure 8A).

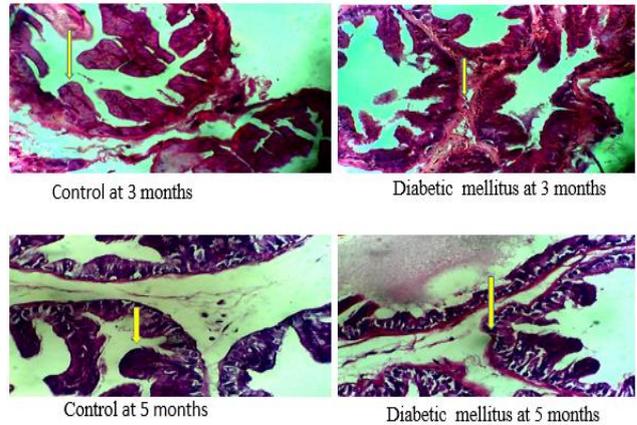


Figure 8. Changes in histology of prostate gland in 3 and 5 months model of STZ diabetic rats (X400). Control group at 3 months DM showing normal architecture; Diabetic group at 3 months DM showing distortion, profuse glandular proliferation with budding of epithelia; Control group at 5 months DM showing normal architecture; Diabetic group at 5 months DM showing distortion, hyperplasia with intraluminal budding of epithelia cells.

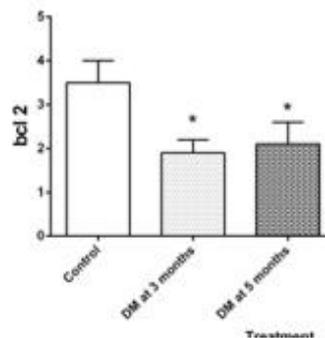
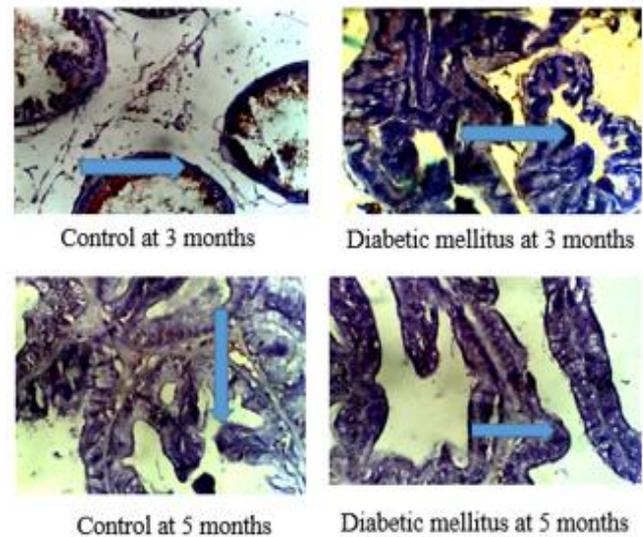


Figure 9. Expression of Bcl₂ protein in prostate of rats after 3 and 5 months induction of diabetes by STZ. *Significantly different when compare with control

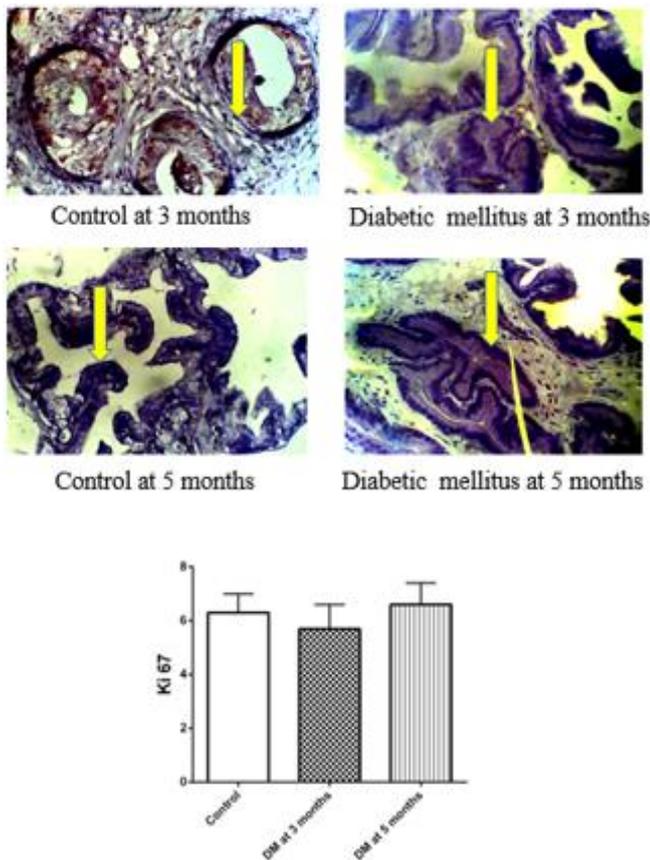


Figure 10. Expression of Ki-67 protein in prostate of rats after 3 and 5 months induction of diabetes by STZ.

Furthermore, immunohistochemical findings revealed significant decrease in the expression of Bcl-2 (Figure 9) while mild expression of Ki67 (Figure 10) in the prostate of 3- and 5-months diabetic rats relative to controls.

DISCUSSION

Streptozotocin-induced DM is characterized by suppression of reproductive activity due to hyperglycaemia-induced oxidative stress and histopathological alterations in testes. Therefore, one of the symptoms of STZ intoxication is hyperglycaemia leading to short and long term complications (Pacher and Szabo, 2005). It is well known that DM-induced hyperglycaemia leads to oxidative stress caused by impairment of mitochondrial electron transfer and activation of the polyol pathway resulting in the excessive generation of reactive oxygen species (ROS) (Ceriello, 2003). In both 3- and 5-months DM studied, prostatic lipid peroxidation products (malondialdehyde) was elevated and this observation is in consonance with the report of Chen et al., (2015). It has been suggested that the level of lipid peroxidation is regulated by enzymatic and non-enzymatic antioxidant system, and scavenger systems whose levels are altered in DM. The depletion of endogenous antioxidant capacity aggravates oxidative stress in DM (Pérez-Matute et al., 2009). In this study, both enzymatic (SOD, CAT and

GPx) and non-enzymatic (GSH) antioxidants levels were reduced and, these results are consistent with previous studies (de Oliveira et al., 2016; Samout et al., 2016). Many research studies focused on glycemic control and common diabetic complications such as diabetic cardiomyopathy, nephropathy, skin ulcer, neuropathy, etc., (Siddiqui et al., 2013; Enomoto et al., 2016). The glycemic control and diabetic related complications in this study were assessed by the levels of plasma fasting glucose, percentage of glycated Hb, urea and creatinine. Oxidative stress, which play a pivotal role in diabetes and its complications, may be the cause of increase in the number of micronucleated polychromatic erythrocytes in the bone marrow of diabetic rats observed in this study. This observation is consistent with the report of Toneline et al., (2014). However, there were no significant differences observed in the percentages of DNA fragmentation in the prostate of diabetic rats using the DPA assay, which is non-specific, thus indicating that apoptotic process in diabetic rats was not disrupted.

It is now known that DM has detrimental effects on male fertility and testis has been the point of emphasis (Abdelali et al., 2016). Diabetes mellitus is considered to produce erectile dysfunction, retrograde ejaculation and reduced levels of testicular hormone and seminal quality changes (De et al., 2016; Al-Roujeaie et al., 2016). In this study, the levels of reproductive hormones; LH, FSH and testosterone were reduced in 3- and 5-months DM rats. This observation is consistent with the study of Cai et al., (2000) who reported that serum testosterone impairment may be linked to varying degrees of testicular and epididymal structural lesions caused by STZ-induced DM. Another laudable explanation is that diabetes may suppress the Leydig cell activity leading to reduced testosterone levels. Also, reduction in testosterone levels may be due to low LH levels in diabetic rats (Kianifard et al., 2013). Decreased testosterone production, on the other hand, may inhibit development of male sex accessories, including growth of the prostate gland (Gilad et al., 1998). In addition, reduced weight of prostate, changes in pH of the prostatic fluid and accumulation of zinc, as observed in this study are factors that suggest marked inhibition of cell proliferation and increased apoptosis in the prostate (Melloul et al., 2002) which correlates with our immunohistochemical staining for Ki67, and Bcl₂ proteins in the prostate of DM rats. The Bcl₂ protein plays a crucial role in the regulation of apoptosis while Ki-67 serves as a marker of proliferative activity of the cells. It has also been reported that complications from DM may alter the mechanism that regulate reactive stroma biology in prostate anatomically, pathologically and / or biochemically (Zinman et al., 2003). Also, alteration in prostatic volume may aggravate components of metabolic syndrome associated with DM (Gacci et al.,

2013). Therefore, pathophysiologic mechanism to explain the changes in metabolic syndrome of DM may include the alterations in prostatic functions, reduction in reproductive hormones and induction of oxidative stress (Sarma and Kellogg, 2009).

Age related decline in testosterone as observed in this study can be attributed to reduction in testosterone production by the testes and reduced hypothalamic secretion of gonadotropin-releasing hormone, which results in inadequate luteinizing hormone secretion by the pituitary gland. Down regulation of the Leydig cell as a result of aging has been linked with ROS-induced damage which in turn reduces the activities of the enzymatic antioxidants superoxide dismutase, glutathione peroxidase and glutathione (Cao et al, 2004; Chen et al, 2009). Histologically, distortion, hyperplasia with intraluminal budding of epithelia cells of prostate was observed, which corroborates our biochemical findings: (Elevation of prostatic MDA, Zn and bicarbonate ions, and reduction in activity of prostatic ACP).

In conclusion, both 3- and 5-months model of DM revealed extensive biochemical and histological changes leading to reduction in prostate weight, level of reproductive hormones, decreased expression of Bcl₂ and accumulation of zinc in prostate. These alterations may ultimately lead to decrease in fertility of the diabetic rats.

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