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Original artilcle

PPAR γ agonist pioglitazone partially reverses hyperglycemia but improves semen quality and testicular histomorphometrics in alloxan-induced diabetic rats

AKINOLA, O.*, SANUSI, S. A., AJAYI, T. F. and OLAJIDE, T. H.

Department of Anatomy, College of Health Sciences, University of Ilorin (Molecular Endocrinology and Neuroscience), Ilorin [[Q1: Q1]], Nigéria

*E-mail: woleakinola@yahoo.com

Abstract

Introduction: Chronic hyperglycaemia is associated with subfertility and infertility in male diabetic subjects, possible as a result of perturbed testicular antioxidant defence system. Recent evidence however shows that some testicular cells produce insulin and this raises the possibility of a role for this hormone in spermatogenesis. Moreover, isoforms of the nuclear peroxisome proliferator activated receptor (PPAR) have been detected in testicular tissue, suggesting a role for insulin sensitization in testicular function. **Materials and Methods:** In this study, we report the ameliorative effects of the PPAR γ agonist pioglitazone on testicular morphologic aberrations and impaired semen profile induced by diabetes in rats treated with intraperitoneal alloxan (150 mg/kg). Diabetic rats were randomly assigned to receive oral pioglitazone at 10 or 30 mg/kg once daily for 6 weeks. Weekly blood glucose measurement using the glucometer showed weak effect of pioglitazone on hyperglycaemia in our model. **Results:** Nonetheless, findings from caudal epididymal sperm analysis showed significantly high sperm density in the pioglitazone-treated rats at the two doses tested ($P < 0.05$ compared with diabetic controls). Moreover, pioglitazone at 10 mg/kg improved sperm motility and lowered the percentages of deformed and dead sperm cells in the treated diabetic rats. Haematoxylin and eosin (H&E) sections of the testes showed significantly reduced seminiferous tubule diameter and lumen size, as well as diminished height of the germinal epithelium in the untreated diabetic rats, but not in rats on pioglitazone intervention. Furthermore, Gordon and Sweet section of the testes showed thickened interstitial compartment only in the untreated diabetic group. Meanwhile, interstitial cells of Leydig appeared undisturbed in testicular sections of all the groups, while intratesticular testosterone levels measured by the enzyme immunoassay technique were not significantly different between the treated and control rats ($P > 0.05$). These findings show that increased sensitization of testicular cells to insulin signalling mediated by the PPAR γ agonist pioglitazone restores testicular histomorphometry and improves semen quality in diabetic rats, despite the weak effect of this drug on blood glucose. **Conclusion:** This suggests a role for insulin sensitizers and improved insulin signalling in the amelioration of testicular lesions and regulation of spermatogenesis in the diabetic state; and lends support for insulin at promoting testicular functions.

Keywords: diabetes mellitus, hyperglycaemia, male infertility, pioglitazone, PPAR gamma.

1 Introduction

Diabetes mellitus arising from either insulin deficit (type 1 diabetes) or insulin resistance (type 2 diabetes) is associated with male reproductive dysfunction (LA VIGNERA, CONDORELLI, VICARI et al., 2012). Recent bodies of evidence from human studies show that diabetes can perturb spermatogenesis by significantly reducing sperm density, sperm motility and progressivity, and volume of the ejaculate, while increasing the incidence of deformed sperm cells (BHATTACHARYA, GHOSH, NANDI, 2014).

Previous animal and human studies have contributed to our present understanding of the pathophysiology of diabetes-induced male subfertility and infertility. Obese men with insulin resistance present with low levels of serum total testosterone, free testosterone and sex hormone binding globulin, resulting from decreased pulse amplitude of luteinizing hormone and inhibitory effects of oestrogens on the hypothalamus. Moreover, oxidative stress, characterized by hyperglycaemia-induced increased generation of reactive oxygen species and weakened antioxidant levels, is a major promoter of testicular damage in diabetes, as a result of sperm nuclear and mitochondrial DNA damage (KILARKAJE and AL-BADER, 2014; ARMAGAN, UZ, YILMAZ et al., 2006). This is corroborated by findings that treatment with melatonin, enalapril, green tea and curcumin could protect against or reverse testicular damage induced by diabetes mellitus (ARMAGAN, UZ, YILMAZ et al., 2006; KANTER, AKTAS and ERBOGA, 2013; KAPLANOGLU, BAHCELIOGLU, GOZIL et al., 2013; KUSHWAHA and JENA, 2012). Besides, sperm nuclear DNA damage is also increased in diabetes as a result of increased levels of advanced glycation end products (AGEs) and increased expression of receptors for advanced glycation end products (RAGEs) in germ and somatic testicular cells (MALLIDIS, AGBAJE, ROGERS et al., 2007). Findings from recent studies by Koh (2007) in streptozotocin (STZ) diabetic rats also showed loss of germ cells by apoptosis mediated by increased expression of Bax, phospho-JNK, and activated caspase in these cells.

Emerging evidence shows that insulin is produced locally by testicular Sertoli cells (GOMEZ, BALLESTER, ROMERO et al., 2009; SCHOELLER, ALBANNA, FROLOVA et al., 2012) and that isoforms of the peroxisome proliferator activated receptors (PPAR) are expressed in testicular cells (including Leydig cells, Sertoli cells and germ cells) (FROMENT, GIZARD, DEFEVER et al., 2006). These point to a significant role for insulin and insulin sensitizers in male reproductive functions. Specifically, PPAR γ plays essential roles in spermatogenesis via yet unknown mechanisms (FROMENT, GIZARD, DEFEVER et al., 2006). Recently, Gumieniczek, Hopkala and Zabek (2008) and Rabbani Devi and Khanam (2010) suggested an antioxidant role for the PPAR γ agonist (pioglitazone) in animal model of diabetes. Pioglitazone at 10 mg/kg body weight increased sperm count and reduced the percentage of deformed sperm cells by reducing malondialdehyde (MDA) levels and enhancing the activity of reduced glutathione and glutathione peroxidase in diabetic rats (RABBANI, DEVI and KHANAM, 2010). Moreover, pioglitazone improved the antioxidant defence system of the testis in alloxan-induced diabetic rabbits without affecting blood glucose levels (GUMIENICZEK, HOPKALA and ZABEK, 2008).

However, it is unclear if pioglitazone treatment could rescue impaired seminiferous tubule morphometry and perturbed semen quality associated with the diabetic state. In recent studies, histomorphometric analysis done on the testicles of diabetic rodents have shown reductions in seminiferous tubule diameter and germinal epithelium thickness, as well as increased thickness and oedema of the interstitial compartment (KIANIFARD, SADRKHANLOU and HASANZADEH, 2012; KHANESHI, NASROLAHI, AZIZI et al., 2013).Thus, in our model, we analyzed testicular histomorphometry and report semen parameters in alloxan-induced Wistar rats with and without oral pioglitazone intervention.

2 Materials and Methods

2.1 Chemicals

Alloxan is a product of Sigma-Aldrich (USA). Pioglitazone is a product of Micro Carsyon (India) and was procured from Aromokeye Pharmacy, Ilorin. Other chemical were of analytical grade and were procured locally.

2.2 Animals

Adult male Wistar rats (9 weeks of age) were sourced from John Alfred Animal Holdings, Ilorin, and were acclimatized to the laboratory condition of 12 hour light:12 hour dark photoperiod and room temperature of 26-30 °C. Rats were placed on standard rodent chow and water was served freely. Care and handling of animals conformed to international standards, according to the National Academy of Sciences' Guide for the Care and Use of Laboratory Animals.

2.3 Induction of hyperglycaemia

Hyperglycaemia was induced in fasted young adult rats by intraperitoneal injection of alloxan monohydrate at 150 mg of alloxan per kg body weight (150 mg/kg) in normal saline. At one week post-alloxan injection, fasting blood glucose was estimated by the glucose oxidase method using a hand-held glucometer (Lifescan, USA). A fasting blood glucose concentration of 250 mg/dl was taken as hyperglycaemic and all induced animals with a minimum of 250 mg/dl of fasting blood glucose were included in the study.

2.4 Oral pioglitazone treatment to diabetic rats and blood glucose assessment

Diabetic rats were randomly assigned to receive normal saline (diabetic controls) or oral doses of pioglitazone (10 mg/kg or 30 mg/kg) by gavage, at 7:00-9:00 daily for six week. Fasting blood glucose levels of the non-diabetic control rats and diabetic rats with and without pioglitazone intervention were estimated weekly, using a glucometer (Lifescan, USA).

2.5 Euthanasia of rats and collection of biological samples

At the end of the 6-week pioglitazone treatment, animals were placed under light anaesthesia with ether. The left testicles were harvested, weighed and homogenized in phosphate buffer (0.1 M, pH 7.4) for intratesticular testosterone assay. Animals were then perfused with saline and then 4% paraformaldehyde (PFA) solution. Perfused right testicles were transferred into 4% PFA for photomicroscopy.

2.6 Intratesticular testosterone assay

Testicular homogenates were centrifuged at 10,000 rpm and the supernatants were assayed for testosterone by the enzyme immunoassay (EIA) technique using the testosterone EIA kit from Cayman Chemical (MI, USA).

2.7 Testicular photomicroscopy and histomorphometric analysis

Paraformaldehyde-fixed testicles were sectioned at 7 µm with the aid of the rotary microtome and then processed for light microscopy by the haematoxylin and eosin (H&E) and Gordon and Sweet (G&S) techniques, as described in Bancroft and Stevens (1982). Using ImageJ software (NIH), haematoxylin and eosin sections of the testes were subjected to morphometric analysis. In the control and diabetic rats, the diameter (external diameter) of the seminiferous tubules, height (thickness) of the seminiferous epithelium and diameter of seminiferous tubule lumen were estimated to the nearest µm.

2.8 Sperm analysis

Sperm from the caudal epididymides was analysed for motility, density, viability and morphology. Sperm count was estimated with the aid of the Neubauer improved cell-counting chamber. The ratio of the motile to non-motile sperm cells (sperm motility) was estimated in all the experimental groups. In addition, the total number of sperm cells with normal morphology was estimated and expressed as a percentage of the total number of sperm cells. The life-death ratio of the sperm cells was also estimated as the number of live sperm cells divided by the total number of sperm cells multiplied by 100.

2.9 Statistical analysis

Data were analysed using one-way analysis of variance (ANOVA), with the aid of the SPSS software version 20 (IBM, USA). Results are presented as mean ± standard error of the mean (mean ± SEM). The means of the variables measured among the groups were compared using Tukey *post hoc* test. P value less than 0.05 ($p<0.05$) was taken as statistically significant. All graphs were drawn using the GraphPad Prism (GraphPad Software Inc., USA).

3 Results

3.1 PPAR γ pioglitazone had weak ameliorative effects on alloxan-induced hyperglycaemia in diabetic rats

Oral pioglitazone treatment at 10 mg/kg and 30 mg/kg partially reversed hyperglycaemia induced by alloxan in adult Wistar rats. Pioglitazone treatment was more effective at lowering high blood glucose at a dose of 10 mg/kg. Though at this dose, blood glucose concentrations remained relatively higher than levels in the untreated non-diabetic rats throughout the 6-week treatment period, but the differences were not statistically significant (P>0.05; Figure 1). Furthermore, diabetic rats treated with pioglitazone at 30 mg/kg remained hyperglycaemic, with blood glucose levels being significantly higher (P<0.05) than those in the untreated non-diabetic rats (Figure 1).

3.2 Semen quality improved in diabetic rats treated with the ppar γ agonist pioglitazone

In the treated alloxan-induced diabetic rats, the PPAR γ agonist (pioglitazone) improved caudal epididymal semen quality at 6 weeks of pioglitazone treatment. In contrast with the untreated diabetic rats where significant decreases in sperm count and motility were observed (P<0.05), no significant differences occurred in these sperm parameters (count and motility) in the diabetic rats treated with pioglitazone at 10 mg/kg compared with the non-diabetic controls (P>0.05). Similarly, the percentages of morphologically normal and viable sperm cells were not different significantly between non-diabetic controls and diabetic rats on 10 mg/kg pioglitazone, but were higher than the values in the untreated diabetic rats (Figure 2).

3.3 Pioglitazone intervention ameliorates impaired testicular morphometry in chronic alloxan-induced hyperglycaemia

In chronic alloxan-induced hyperglycaemia, there were significant reductions (P<0.05) in the height (thickness) of the germinal epithelium, diameter of the seminiferous tubule and size of the seminiferous tubule lumen compared with the non-diabetic controls (Figure 3). Meanwhile, in diabetic rats on pioglitazone intervention, these testicular histomorphometric parameters increased significantly (P<0.05) in comparison with the diabetic controls.

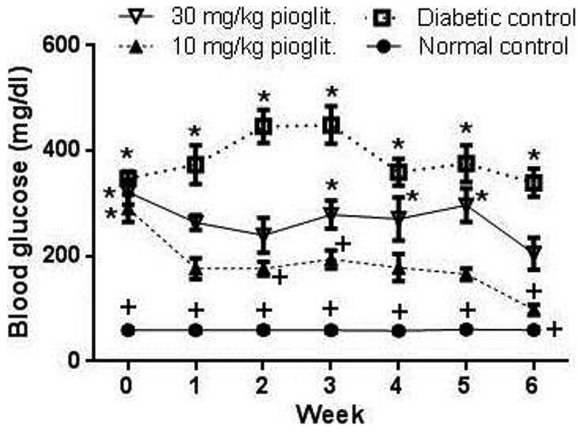

Figure 1. Blood glucose concentrations in non-diabetic controls and in diabetic rats with or without pioglitazone treatment over a 6-week period. Hyperglycaemia in induced diabetic rats was partially reversed by oral pioglitazone treatment. Data are mean ± SEM. *Statistically significant at P<0.05 compared with non-diabetic controls; +Statistically significant at P<0.05 compared with diabetic controls.

3.4 Testicular weight and intratesticular testosterone levels are not different between experimental groups

Relative testicular weights and intratesticular testosterone levels were not different significantly between the non-diabetic controls and diabetic rats with and without pioglitazone treatment (Figures 3d and 4)

3.5 PPAR-γ agonist pioglitazone protects against testicular damage resulting from chronic hyperglycaemia in alloxan diabetic rats

Chronic hyperglycaemia resulted in marked loss of maturing germ cells in the seminiferous tubule walls in alloxan-induced diabetic rats (Figure 5). Haematoxylin and eosin sections of the testicles showed evidence of spermatogenesis arrest and loss of most spermatids and spermatozoa, as suggested by the void adluminal compartment of the seminiferous tubule in the untreated diabetic rats (Figure 5B). Moreover, paucity of spermatocytes is also observable. The only type of germ cells that remained relatively unaffected by chronic alloxan-induced hyperglycaemia was the spermatogonial stem cells. Pioglitazone treatment to diabetic rats at 10 mg/kg and 30 mg/kg however protects against testicular lesions induced by chronic hyperglycaemia (Figure 5C, D). Testicular histology in these treated groups showed the presence of spermatids and spermatocytes in the germinal epithelium, as well as spermatozoa in the adluminal compartment of the seminiferous tubule compared with testicular morphology in the untreated diabetic control (Figure 5B). Moreover, testicular sections in the latter showed thickening of the interstitial tissue between adjoining seminiferous tubules, as demonstrated by G&S staining (Figure 6B). Such thickening of the interstitium, characterised by increased reticular fibre mass, was not observed in the pioglitazone-treated diabetic rats (Figure 6C, D).

4 Discussion

We report testicular morphometry and caudal epididymal sperm parameters in adult alloxan-induced diabetic Wistar rats treated with oral doses of pioglitazone, an agonist for the nuclear peroxisome proliferator-activated receptor gamma (PPAR γ). Pioglitazone partially reversed hyperglycaemia induced by alloxan in adult Wistar rats. The treatment was more effective at 10 mg pioglitazone/kg body weight (10 mg/kg pioglitazone). Rats on 30 mg/kg pioglitazone showed significantly elevated blood glucose concentrations compared with the untreated non-diabetic rats during the 6-week treatment period. The partial reversal of hyperglycaemia by pioglitazone might be associated with improved sensitivity to insulin in peripheral tissues such that the insulinopenic state of alloxan diabetic rats was compensated for by increased sensitivity to insulin especially in adipocytes, but also in skeletal and cardiac myocytes, liver cells and activated macrophages (SCHOONJANS and AUWERX, 2000). Enhanced insulin signalling in these cells could mediate increased glucose disposal, leading to the observed weak effects on glycaemia

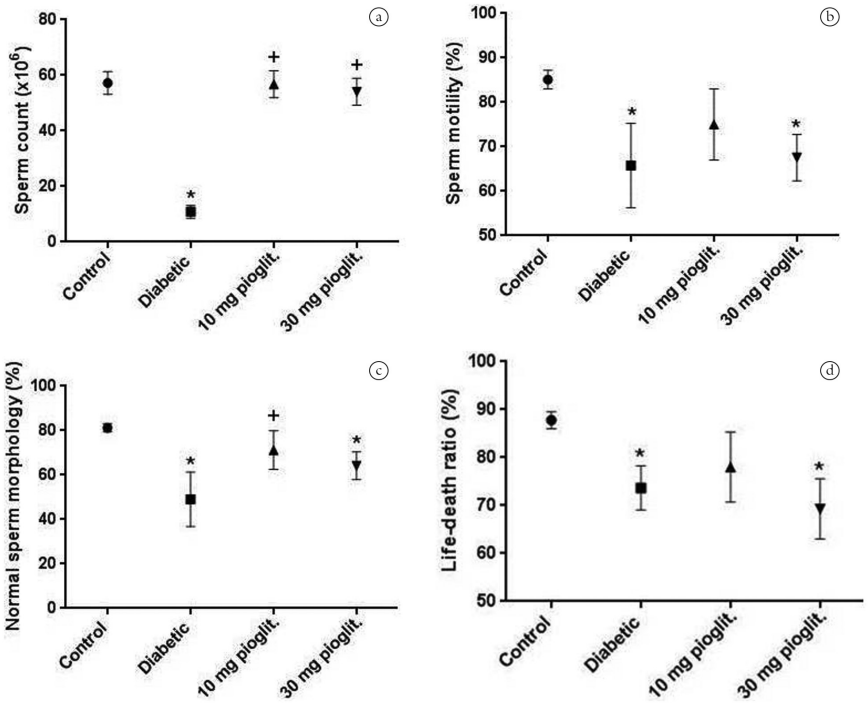

Figure 2. Caudal epididymal sperm profile in the different experimental groups. Sperm count (a), sperm motility (b), percentage of morphologically normal sperm cells (c) and percentage of viable sperm cells (d) are not significantly different between non-diabetic controls and diabetic rats on 10 mg/kg pioglitazone. These parameters are however markedly reduced in the untreated diabetic rats. Data are mean ± SEM. *Statistically significant at P<0.05 compared with non-diabetic controls; +Statistically significant at P <0.05 compared with diabetic controls.

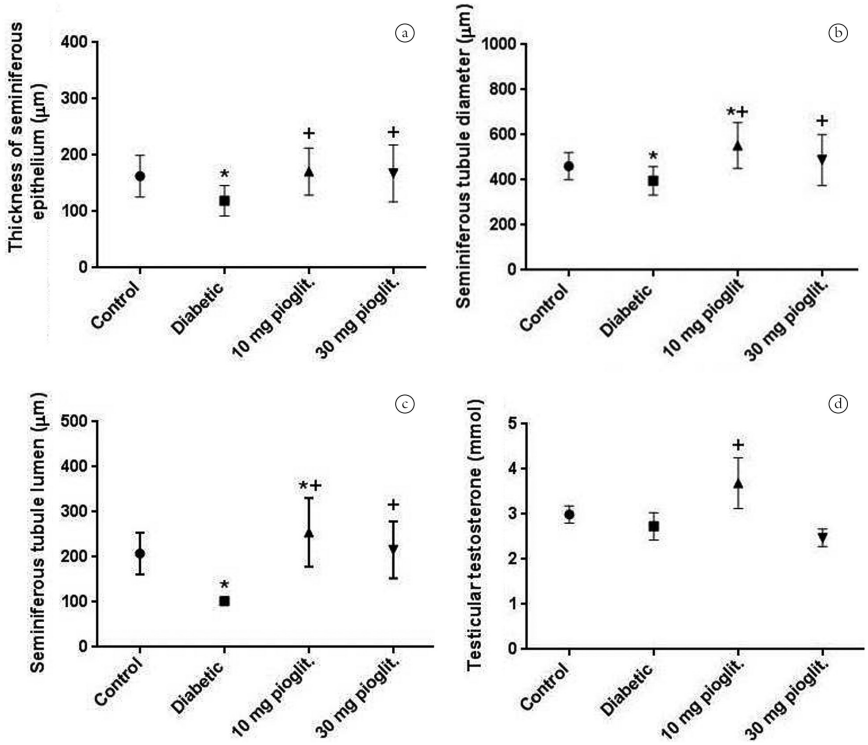

Figure 3. Pioglitazone treatment significantly improved testicular histomorphometrics, including the thickness of the seminiferous epithelium (a), diameters of the seminiferous tubule (b), and size of the seminiferous tubule lumen (c), compared with the diabetic controls. Moreover, intratesticular testosterone levels (d) are not significantly different between the non-diabetic controls and the treated or untreated diabetic rats. *Statistically significant at P<0.05 compared with non-diabetic controls; +Statistically significant at P<0.05 compared with diabetic controls.

in our model. This agrees with findings in rabbits in which pioglitazone did not produce any significant changes in blood glucose levels in the treated animals (GUMIENICZEK, HOPKALA and ZABEK, 2008).

Thus, the improved testicular histology and morphometry mediated by pioglitazone in our alloxan diabetic rat model was not consequent upon normalization of blood glucose, but molecular events other than reversal of hyperglycaemia was largely responsible.

As indicated in Figure 3, chronic hyperglycaemia induced by alloxan in Wistar rats resulted in significant reductions in lumen size and external diameters of the seminiferous tubules, as well as in diminished height of the germinal epithelium; although relative testicular weight did not differ significantly between the untreated diabetic and non-diabetic controls. Related findings have been reported supporting impaired testicular morphometry in diabetic animals. In diabetic rats induced with streptozotocin (STZ), Kianifard, Sadrkhanlou and Hasanzadeh (2012) and Khaneshi, Nasrolahi, Azizi et al. (2013) observed reductions in the diameters of the seminiferous tubules and height of the seminiferous epithelium in the induced diabetic state. These and findings in our study lend morphometric support to the poor semen quality observed in the diabetic rats in our rodent model.

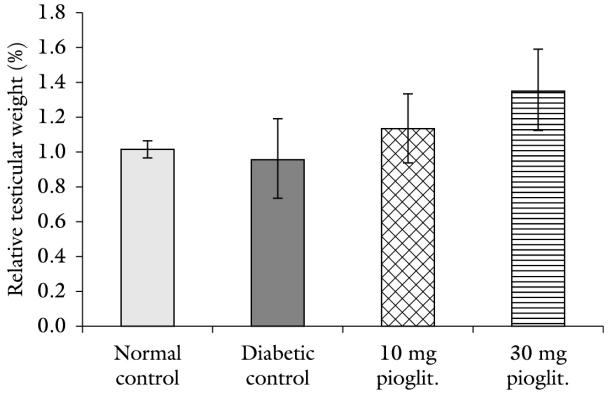

Figure 4. Relative testicular weights of the treated and control diabetic rats. There are no significant differences in relative testicular weights between the groups (P>0.05).

Semen quality was adversely affected by alloxan-induced diabetes in rats (Figure 2). Significantly low sperm count and motility, as well as low number of morphologically normal and viable sperm cells were pointers to the deleterious impact of diabetes. Interestingly, oral pioglitazone at 10 mg/kg improved semen quality in these rats. However, apart from sperm density that did not differ significantly from the non-diabetic controls, other sperm parameters were not ameliorated by treatment of diabetic rats with pioglitazone at 30 mg/kg body weight. Meanwhile, it is unclear from our study how treatment of diabetic rats with pioglitazone at 10 mg/kg resulted in improved semen profile and testicular histomorphometry in these animals. Nonetheless, it is known that glitazones, acting as agonists for PPAR γ could attenuate hepatic oxidative stress-mediated DNA damage in high fat diet-induced obese mice, suggesting that this drug could improve antioxidant defence system (HSIAO,

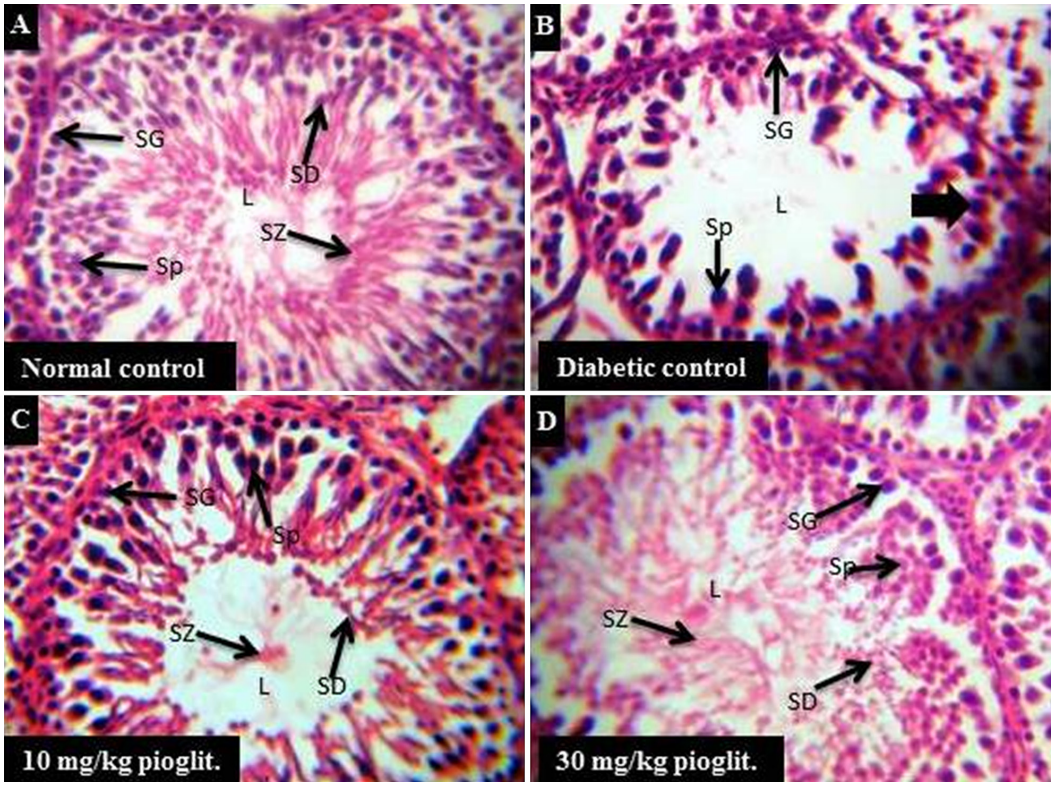

Figure 5. Representative photomicrographs of the testicles of the treated and untreated diabetic rats. Attenuation of the seminiferous epithelium, characterized by loss of spermatozoa (SZ) in the adluminal compartment (L); and diminished spermatids (SD) and spermatocytes (Sp), is observable in the untreated diabetic group (B), but absent in the non-diabetic controls (A); and in pioglitazone-treated diabetic rats (C, D). H&E stain; x400.

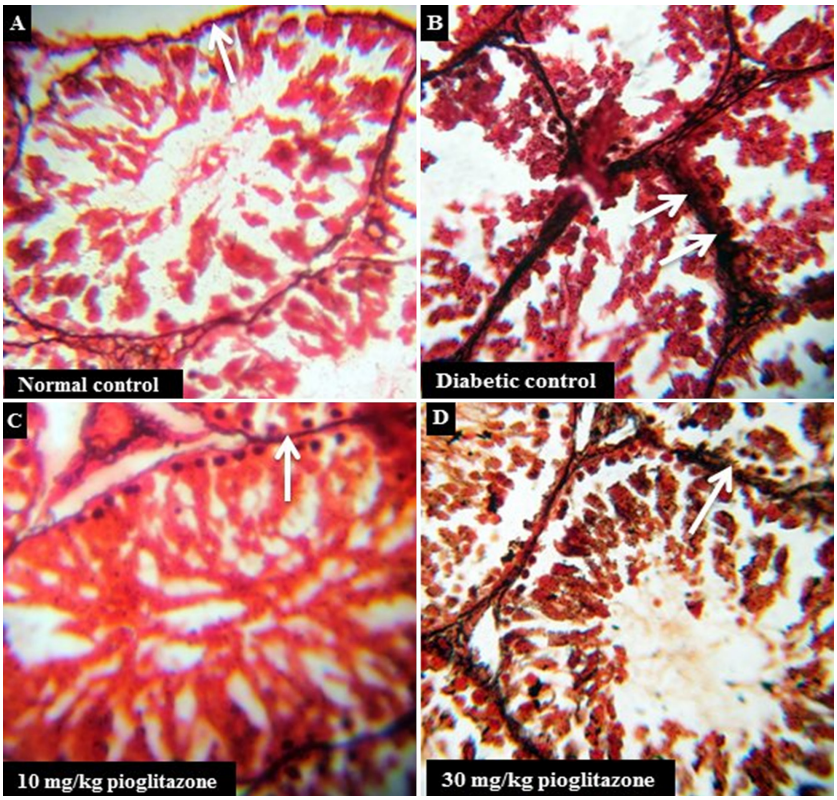

Figure 6. Representative photomicrographs of Gordon and Sweet-stained testicular sections of rats. Reticular fibres mass and interstitial matrix (arrows) have increased in the untreated diabetic rats (B), but not in the normal controls (A) or diabetic rats on pioglitazone intervention (C, D). x400.

HSIEH, KUO et al., 2008). Moreover, in human neuron-like NT2 cell line, pioglitazone increased mitochondrial DNA content and reduced mitochondrial peroxide levels (GHOSH, PATEL, RAHN et al., 2007). In addition, a related glitazone, troglitazone, has been shown to possess differential effects on retinal pigmented epithelium survival in response to oxidative stress (RODRIGUES, MAURIER-MAHE, SHURLAND et al., 2011). Furthermore, pioglitazone reduced NADPH oxidase activity and superoxide anion generation in obese diabetic mice (HWANG, KLEINHENZ, RUPNOW et al., 2007). In human coronary artery endothelial tissue, Mehta, Hu, Chen et al. (2003) showed that pioglitazone treatment could reduce superoxide radical generation. Interestingly, Gumieniczek, Hopkala and Zabek (2008) have recently showed that treatment of alloxan-induced diabetic rabbits with pioglitazone produced increased activity of antioxidant enzymes including catalase, superoxide dismutase, glutathione peroxidase, and glutathione reductase, as well as elevated glutathione (GSH) levels. These findings are pointers to the potential of glitazones at improving antioxidant capacity in different tissue types.

Although we did not evaluate testicular oxidative stress in our model, but improved antioxidant defence system, mediated by pioglitazone treatment, might partially account for the enhanced histological features and improved sperm parameters in our model. Interestingly, in addition to expressing PPAR α and PPAR β/δ, testicular germ and somatic cells also express PPAR γ (FROMENT, GIZARD, DEFEVER et al., 2006), thereby raising the possibility of a direct effect of pioglitazone on these cells.

Furthermore, a close assessment of the H&E-stained testicular sections in the untreated diabetic rats showed gross erosion of germinal epithelial cells. Spermatogonia were the only germ cell type that were relatively undisturbed by the alloxan-induced diabetic state in these rats; but spermatozoa are almost completely undetectable in the adluminal compartment, while spermatids and spermatocytes are markedly diminished (Figure 5). These histologic findings agree with the testicular morphometric data and further support the poor quality of the caudal epididymal sperm in the untreated diabetic rats. On the contrary, testicular histologic findings and caudal epididymal sperm profile in diabetic rats on pioglitazone intervention suggest reversal of testicular lesions, especially in diabetic rats treated with 10 mg/kg pioglitazone (Figures 2 and 5). In the latter group, blood glucose levels were higher but not significantly different from the non-diabetic control rats (Figure 1). However, since testicular histology and morphometry likewise improved in diabetic rats administered pioglitazone at 30 mg/kg, in which blood glucose levels were not significantly different from the untreated diabetic controls, it is appropriate to suggest that mechanisms other than amelioration of hyperglycaemia were responsible for the improvement of testicular histologic lesions by pioglitazone (at 30 mg/kg body weight).

It is therefore possible that a functional relationship exists between modulation of insulin sensitivity by agonists and ligands of PPAR γ on one hand, and regulation of spermatogenesis on the other. In support of this hypothesis are findings from animal studies showing that insulin is expressed in testicular spermatids and Leydig cells, and it is essential for the regulation

of testicular function and spermatogenesis (GONDOS and BEVIER, 1995; LOEKEN, 2012); and that exogenous insulin could regenerate testicular structure in Akita mouse (SCHOELLER, ALBANNA, FROLOVA et al., 2012). It is therefore plausible to suggest that in the insulin-resistant state of metabolic syndrome and type 2 diabetes or insulin-deficient state of type 1 diabetes, enhanced sensitivity to circulating insulin could promote spermatogenesis and improve male fertility. This is suggested by findings in the present study and the recent discovery of isoforms of the PPAR in testicular cells (FROMENT, GIZARD, DEFEVER et al., 2006). However, the exact interrelationship between testicular insulin levels (from intratesticular or pancreatic source), expression and activity of testicular PPAR γ, and regulation of spermatogenesis, awaits further investigation.

As shown in Figure 6, thickening of the testicular interstitium, characterized by increased reticular fibre mass in Gordon and Sweet-stained sections of the testicles is a finding in alloxan-induced diabetic rats in our model. Meanwhile, no evidence of Leydig cell loss was observed in the interstitium of haematoxylin and eosin-stained testicular sections (Figure 5). Moreover, biochemical assay for intratesticular testosterone levels also showed that this steroid was not significantly altered by the diabetic state when compared with the non-diabetic controls. In related animal and human studies, Bal, Turk, Tuzcu et al. (2011) demonstrated interstitial tissue oedema and congestion of the interstitium in diabetic rats, while Cameron Murray and Drylie (1985) demonstrated increased mass of collagen-rich materials around blood vessels and seminiferous tubules in infertile diabetic men, indicating matrix expansion. Thus, from our findings and those of others, hyperglycaemia is associated with interstitial matrix expansion in the diabetic state.

The absence of significant differences in intratesticular testosterone levels between the diabetic and non-diabetic control groups suggest that the histomorphometric and sperm profile alterations in the testicles of diabetic rats was not a consequence of low testosterone, and that factors other than hypogonadism are responsible. Findings in STZ-induced hyperglycaemic mice showed low epididymal sperm density in the presence of normal testosterone levels (BOSE, ADIGA, D'SOUZA et al., 2012). These further support the hypothesis that in the diabetic state, testicular lesions may occur in the presence of normal levels of intratesticular and serum testosterone.

5 Conclusion

Put together, findings in our alloxan model of diabetes show that pioglitazone treatment to insulinopenic hyperglycaemic rats improves semen profile and ameliorates altered testicular histomorphology possibly as a result of enhanced insulin sensitization mediated by the PPAR γ agonist pioglitazone, even in the presence of chronic, relatively high blood glucose concentrations. This supports specific roles for insulin and enhanced insulin sensitization by PPAR agonists in the regulation of spermatogenesis and testicular functions in rodents.

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