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Streptozotocin diabetes and insulin resistance impairment of spermatogenesis in adult rat testis: central Vs local mechanism

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Summary: Mammalian reproduction is dynamically regulated by the pituitary gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH). These hormones are synthesized in the pituitary gland following stimulation by the gonadotropin-releasing hormone (GnRH) and act by stimulating steroid production and gametogenesis in both males and females. Male adult Sprague-Dawley rats (120 – 140 g) were randomly divided into 7 groups. Group 1 > Control group; fed on normal rat pellets. Group 2 > Streptozotocin group; received a single dose IP injection of streptozotocin 45 mg/kg BW in Na^+ citrate buffer pH 4.5. Group 3 > Streptozotocin-insulin treated group; received a single dose IP injection of streptozotocin as in group 2 above and treated with insulin sub-cutaneously. Group 4 > Streptozotocin-ginger treated group; received a single dose IP injection of streptozotocin as in group 2 above and treated with 500 mg/Kg Ginger extract orally. Group 5 > Insulin resistant group; fed ad libitum on a special diet containing 25% fructose mixed with 75% normal rat chow (w/w). Group 6 > Insulin resistant-pioglitazone treated group; fed ad libitum on a special diet as in group 5 above and treated with Pioglitazone 15 mg/kg orally. Group 7 > Insulin resistant-ginger treated group; fed ad libitum on a special diet as in group 4 above, and also treated with 500 mg/Kg Ginger extract orally. Hormonal and tissue biochemistry analyses revealed that both central and local mechanisms are implicated in the impairment of spermatogenesis by diabetes but the hypothalamo-pituitary testicular axis alteration might not likely have a major impact as the local defect on steroidogenesis in the testis. This local defect could also predispose to male hypogonadism, i.e. failure of gonadal function.

Keywords*:* Diabetes mellitus, Insulin resistance, LH, FSH, Testosterone, Cholesterol.

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INTRODUCTION

Mammalian reproduction is dynamically regulated by the pituitary gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH). These hormones are synthesized in the pituitary gland following stimulation by the gonadotropin-releasing hormone (GnRH) and act by stimulating steroid production and gametogenesis in both males and females.

FSH acts on the Sertoli cells of the testes and is involved in spermatogonial maturation (Haywood et al., 2003; Meachem et al., 2005). It stimulates primary spermatocytes to undergo the first division of meiosis, to form secondary spermatocytes. It also enhances the production of androgen-binding protein by the Sertoli cells of the testes by binding to FSH receptors on their basolateral membranes (Boulpaep and Boron, 2005) and is critical for the initiation of spermatogenesis. LH on the other hand triggers the production of testosterone in the Leydig cells. Testosterone is essential for spermatid elongation and development of secondary sexual characteristics (O'Donnell et al., 1996; O'Donnell et al., 1994).

The spermatogenic cycle is divided into 14 stages in the rat (Leblond and Clermont, 1952; Wing and Kent, 1998); 12 stages in the mouse (Oakberg, 1956; Russell et al., 1990); and 6 stages in humans (Hermo et al., 2010). In the rat, one spermatogenic cycle takes ≈ 12 – 14 days to complete (Dym and Clermont, 1970) and ≈ 8 – 9 days in the mouse (Russell et al., 1990).

Studies have shown that it takes ≈ 35 to 54 days for a single spermatogonium to complete spermatogenesis and give rise to eight haploid spermatids in the rat **(**Courot et al., 1970; De Krester and Kerr, 1988) and the mouse (Russell et al., 1990) respectively. This is because developing germ cells must go through the cycle 4.5 times before they can become fully developed spermatids (spermatozoa) that are released into the tubular lumen at stage VIII

of the cycle. Furthermore, the biochemical, molecular and cellular events pertinent to spermatogenesis are under endocrine control of the hypothalamic-pituitary-testicular axis (Parvinen, 1982).

There are a number of reports in the literature examining the effects of diabetes on the endocrine control of spermatogenesis (Ballester et al., 2004; Baccetti et al., 2002; Garcia- Diez et al., 1991). However, the results of these studies have been conflicting and the reported abnormalities are unlikely to impair reproductive function significantly in isolation (Sexton and Jarow, 1997).

The paucity of studies addressing the effects of diabetes mellitus on human male reproductive function and the conflicting nature of existing data have resulted in a distinct lack of consensus as to the extent of the problem. Despite this fact, data from animal models strongly suggest that diabetes mellitus affects semen parameters and impairs spermatogenesis in male rats (Arikawe et al., 2006). More recently, Arikawe et al. 2012 reported that streptozotocin-diabetic and insulin resistance reduced testicular PCNA index, mean seminiferous tubular diameter and testicular diameter. Thus this study aimed to assess the central and local mechanisms involved in the impairment of spermatogenesis in diabetic and insulin resistant rat testis.

MATERIALS AND METHODS

Forty-two adult male Sprague-Dawley rats, whose average weight ranged between 120 – 140 g were procured from a breeding stock maintained in the Laboratory Animal Department of the College of Medicine, University of Lagos. The animals were housed in clear polypropylene cages lined with wood chip beddings and were allowed to acclimatize in the Physiology Department animal laboratory with an ambient temperature maintained between 26ºC – 28ºC for a period of one week before the beginning of the study. The rats were also maintained under standard colony photoperiodic conditions with a 12-hour light/12-hour dark cycle (lights on at 7:00 hour) and all animals had unrestricted (ad libitum) access to water. The rats were randomly divided into 7 groups (of 6 animals each of similar weight).

Group 1 rats served as Control group, fed on normal rat chow throughout experimental period of 16 weeks. Group 2 rats served as Streptozotocin diabetic group; fed on normal rat chow and received a single dose IP (intraperitoneal) injection of Streptozotocin, 45 mg/kg body weight (Guneli et al., 2008) freshly dissolved in Na^+ citrate buffer pH 4.5 for 4 weeks. Group 3 rats served as Streptozotocin-insulin treated group; fed on normal rat chow; received a single dose IP (intraperitoneal) injection of Streptozotocin injection as in Group 2 rats above, and also treated with 0.5 – 1 IU Isophane Insulin sub-cutaneously for additional 4 weeks. Group 4 rats served as Streptozotocin-ginger treated group; fed on normal rat chow; received Streptozotocin injection as in Group 2 rats above, and also treated with 500mg daily of ginger extract/Kg body weight orally for additional 4 weeks. This chosen dosage of 500mg ginger extract/kg body weight was previously found to be effective and non-toxic in rats (Thomson et al., 2002; Morakinyo et al., 2008; Alnaqeeb et al., 2003). Blood samples were collected from the tail vein 48 hours after Streptozotocin injection to confirm hyperglycaemia using Dextrostix Test Strips (Bayer Corporation, U. K.) following the glucose oxidase method (Hugget and Nixon, 1957). Streptozotocin administration was not prolonged for more than 4 weeks without treatment because the glycaemic levels were very high, and as time passed the animals became weaker (Watala, 2009) and eventually resulted in mortality.

Group 5 rats served as Insulin resistant diabetic group; fed ad libitum on a special diet containing 25% fructose mixed with 75% normal rat chow (w/w) for 4 weeks (Arikawe and Olatunji-Bello, 2004) and continued till the 12^{th} week (Arikawe et al., 2006). Hyperglycaemia was confirmed at the 12^{th} week using Dextrostix Test Strips (Bayer Corporation, U. K.) following the glucose oxidase method (Hugget and Nixon, 1957). Group 6 rats served as Insulin resistant-pioglitazone treated group; fed ad libitum on a special diet as in group 5 rats above, and also treated with Pioglitazone 15mg/kg orally for additional 4 weeks. Group 7 rats served as Insulin resistant-ginger treated group; fed ad libitum on a special diet as in group 5 rats above, and also treated with 500mg daily of ginger extract/Kg body weight orally for additional 4 weeks. Hyperglycaemia was confirmed at the 16^{th} week using Dextrostix Test Strips (Bayer Corporation, U. K.) following the glucose oxidase method (Hugget and Nixon, 1957).

Polydipsia, polyuria and polyphagia were observed (Jelodar et al., 2010) and confirmed in the experimental groups (groups 2 to 7). All animals had free access to drinking water throughout the duration of the study. Rats with blood glucose concentration above 250 mg/dl were used as Streptozotocin diabetic rats (Akingba and Burnett, 2001), while rats with blood glucose concentration above 200 mg/dl were used as Insulin resistant diabetic rats (Catena et al., 2003). Rats were weighed weekly throughout the duration of the experiment and animals were monitored for general health during the treatment period. All the procedures were performed in accordance with the guidelines of the College Ethical Committee on the use of laboratory animals for research.

METHODOLOGY

At the end of each experimental period, following hyperglycaemia confirmation, the following analyzes were carried out (i) Haematologic analysis for Glycosylated Haemoglobin levels (ii) Hormonal Analysis for the following hormones Insulin, Testosterone, Prolactin, LH, and FSH using Enzyme-linked immunosorbent assay (ELISA) methods (iii) Tissue Biochemistry for Testis (testosterone and cholesterol levels using Enzyme-linked immunosorbent assay (ELISA); Acid phosphatase, Alkaline phosphatase, Lactate dehydrogenase, and Seminal Vesicle (seminal fructose) levels using the colorimetric assay/methods (iv) Histopathological assessments on pancreas.

Haematologic and hormonal analysis

Cervical dislocation was carried out on the animals in all the groups. Blood was quickly collected by cardiac puncture into heparinized bottles; whole blood sample was used to determine the glycosylated haemoglobin levels while the remaining blood samples were centrifuged at 3,000 x g for 15 minutes and the plasma sample was transferred into a new tube, kept frozen (-20ºC) until measurement of the different hormones (Insulin, Testosterone, Prolactin, LH, and FSH) using Enzyme-linked immunosorbent assay (ELISA) methods.

Tissue Biochemistry

Testis:

Testosterone and cholesterol contents were determined in testicular tissue. One testis was crushed in 2 ml of 0.9% NaCl in distilled water with the aid of a homogenizer. The homogenate was centrifuged at 3,000 rpm for 15 minutes. The supernatant was removed and used for determination of testicular testosterone and cholesterol contents using the Enzyme-linked immunosorbent assay (ELISA) used for the plasma samples.

Acid phosphatase, Alkaline phosphatase and Lactate dehydrogenase activities were determined using the colorimetric method.

Seminal vesicle:

Extraction procedures were similar to that of the testis. The homogenate was centrifuged at 3,000 x g for 15 minutes and supernatant used for determination of seminal vesicle fructose.

For qualitative histopathological assessments the pancreas was isolated from the animals, fixed in 10% formalin, passed through ascending series of ethanol baths, embedded in paraffin, mounted on slides and stained with Haematoxylin and Eosin. The slides were subsequently viewed under the light microscope and photomicrographs taken at different magnifications.

Statistical Analysis

All data are presented as mean ± standard error of mean (SEM). The data was analyzed using One-way ANOVA (analysis of variance) followed by Student-Newman-Keuls post-hoc test. Level of statistical significance was taken at $P < 0.05$.

RESULTS

Fasting blood glucose concentration (mg/dl) and Glycosylated haemoglobin level (%) in Control rats was (91.4 ± 2.1mg/dl, 5.5 ± 0.2%); Streptozotocin (520.5 ± 34.2mg/dl, 9.6 ± 0.1%); Streptozotocin-insulin treated (283.4± 13.1mg/dl, 6.6 ± 0.1%); Streptozotocin-ginger treated (423.3± 18.6mg/dl, 6.9 ± 0.2%); Insulin resistant (203.2± 1.3mg/dl, 8.9 ± 0.1%); Insulin resistant-pioglitazone treated (101.5 ± 2.3mg/dl, 7.8 ± 0.3%); and Insulin resistant-ginger

Table 1: Fasting Blood Glucose, Glycosylated Haemoglobin, Insulin (Ins), Prolactin (PRL), LH, FSH, and Plasma Testosterone (Test) in Control, Streptozotocin, Streptozotocin-Insulin, Streptozotocin-Ginger, Insulin resistant, Insulin resistant-Pioglitazone, and Insulin resistant-Ginger groups

	Control	Streptozotocin	Streptozotocin-Insulin	Streptozotocin-Ginger	Insulin resistant	Insulin resistant-Pioglitazone	Insulin Resistant-Ginger
FBG mg/dl	91.4 ±2.1	$520.5\pm34.2^{\dagger\ddagger a*\#}$	$283.4\pm 13.1^{\dagger\ddagger a}$	$423.3\pm 18.6^{\dagger\ddagger a\#}$	$203.2\pm 1.3^{\dagger\ddagger a}$	101.5 ± 2.3	117.1 ± 2.5
$HbA1_c$ (%)	5.5 ± 0.2	$9.6 \pm 0.1^{\dagger\#\ddagger a*}$	$6.6 \pm 0.1^{\dagger}$	$6.9 \pm 0.2^{\dagger}$	$8.9 \pm 0.1^{\dagger\#*\ddagger a}$	$7.8 \pm 0.3^{\dagger\#*}$	$7.8 \pm 0.1^{\dagger\#*}$
Ins (IU/ml)	1.9 ± 0.1	$1.0 \pm 0.1^{\dagger}$	$1.8 \pm 0.2^{\mu\alpha}$	$1.7 \pm 0.1^{\mu}$	$1.6 \pm 0.2^{\dagger\mu}$	$1.5 \pm 0.1^{\dagger\mu\#a}$	$1.7 \pm 0.1^{\mu}$
PRL mIU/L	6.0 ± 0.1	$3.7 \pm 0.05^{\dagger\ddagger}$	$6.0 \pm 0.1^{\mu\alpha\ddagger}$	$8.5 \pm 0.07^{\dagger a\mu\alpha\#\ddagger}$	$3.8 \pm 0.07^{\dagger\ddagger}$	$3.2 \pm 0.1^{\dagger}$	$6.0 \pm 0.1^{\mu*\ddagger}$
LH (IU/L)	0.73± 0.08	$0.17 \pm 0.01^{\dagger}$	$0.20 \pm 0.01^{\dagger}$	$0.57 \pm 0.01^{\dagger a\mu\#*}$	$0.40 \pm .01^{\dagger\#a\mu}$	$0.20 \pm 0.01^{\dagger*\alpha}$	$0.10 \pm 0.01^{\dagger}$
FSH (IU/L)	0.08 ± 0.01	0.09 ± 0.01^{a}	$0.19 \pm .01^{\dagger a\mu\alpha*}$	0.11 ± 0.01^{a}	0.08 ± 0.01	$0.19 \pm 0.01^{\dagger a\mu\alpha*}$	0.06 ± 0.01
Test(nmol/L)	4.9 ± 0.09	$5.9 \pm 0.1^{\dagger\alpha\#a*}$	$4.2 \pm 0.07^{\dagger}$	$3.7 \pm 0.1^{\dagger\#}$	$8.8 \pm 0.1^{\dagger\alpha\#a}$	$5.4 \pm 0.1^{\dagger\alpha\#a\mu*}$	$4.8 \pm 0.1^{\alpha\#}$

All results presented in mean ± SEM $^{\dagger}P < 0.001$ Vs Control; $^{\mu}P < 0.001$ Vs Streptozotocin $^{\#}P < 0.001$ Vs Streptozotocin-Insulin; $*P < 0.001$ Vs Streptozotocin- Ginger; $^{\alpha}P < 0.05$ Vs Insulin resistant; $^{a}P < 0.001$ Vs Insulin resistant-Ginger; $^{\ddagger}P < 0.001$ Vs Insulin resistant- Pioglitazone

Table 2. Testicular testosterone (Test), Cholesterol (Chol), Seminal fructose (Fruc), Alkaline phosphate (ALP), Lactate Dehydrogenase (LDH), Acid phosphatase (AP) in Control, Streptozotocin, Streptozotocin-Insulin, Streptozotocin-Ginger, Insulin resistant, Insulin resistant-Pioglitazone, Insulin resistant-Ginger groups

	Control	Streptozotocin	Streptozotocin-Insulin	Streptozotocin-Ginger	Insulin resistant	Insulin resistant-Pioglitazone	Insulin resistant-Ginger
Test (nmol/L)	11.3 ± 0.2	2.3 ± 0.3†	5.9 ± 0.4$^{\dagger\mu}$	6.4 ± 0.5$^{\dagger\mu}$	5.1 ± 0.5$^{\dagger\mu}$	5.6 ± 0.6$^{\dagger\mu}$	6.6 ± 0.3$^{\dagger\mu}$
Chol (mg/dl)	132.3± 6.2	359.0 ± 4.1$^{\dagger a}$	215.7 ± 8.7$^{\dagger a\mu}$	211.2± 4.7$^{\dagger a\mu}$	232.0±4.9$^{\dagger a\mu}$	220.7± 3.6$^{\dagger a\mu}$	163.3± 6.7†
Fruc (g/L)	4.95 ± 0.46	1.70 ± 0.30†	2.00 ± 0.24†	2.30 ± 0.24†	5.23±0.47$^{\mu\#}$*	5.27 ± 0.38$^{\mu\#}$*	5.67 ± 0.43$^{\mu\#}$*
ALP (IU/L)	36.5 ± 4.8	129.8 ± 9.9†	112.0 ± 7.4†	109.0 ± 3.5$^{\dagger a}$	88.0 ± 4.3$^{\dagger\mu}$	75.3 ± 4.2$^{\dagger\mu}$*$^{\#}$	68.3 ± 4.0$^{\P\mu\#}$
LDH (IU/L)	156.0± 4.2	81.7 ± 11.9†	101.3 ± 7.2†	103.0± 8.0†	91.7 ± 10.5†	104.0 ± 6.0†	105.7 ± 7.7†
AP (IU/L)	0.57 ± 0.07	0.47 ± 0.07	0.63 ± 0.07	0.63 ± 0.07	0.52 ± 0.05	0.63 ± 0.05	0.67 ± 0.05

All results presented in mean ± SEM $^{\dagger}P < 0.001$; $^{\dagger}P < 0.01$ Vs Control; $^{\mu}P < 0.001$ Vs Streptozotocin $^{\#}P < 0.001$ Vs Streptozotocin-Insulin; *P < 0.001 Vs Streptozotocin- Ginger; $^{a}P < 0.001$ Vs Insulin resistant-Ginger

treated rats (117.1 ± 2.5mg/dl, 7.8 ± 0.1%) respectively. These were significantly lower (P < 0.001) in the Control rats compared to rats in the experimental groups with the exception of insulin resistant-pioglitazone and insulin-resistant ginger treated groups (Table 1).

Plasma insulin concentration was significantly higher (P < 0.001) in the Control rats (1.9 ± 0.1 IU/ml) compared to the rats in Streptozotocin (1.0 ± 0.1 IU/ml); Insulin resistant (1.6 ± 0.2 IU/ml) and Insulin resistant-Pioglitazone groups (1.5 ± 0.1 IU/ml). However, its concentration in Streptozotocin-Insulin rats (1.8 ± 0.2 IU/ml); Streptozotocin-Ginger rats (1.7 ± 0.1 IU/ml) and Insulin resistant-Ginger rats (1.7 ± 0.1 IU/ml) was not significantly different to that of the Control rats (Table 1).

Plasma prolactin concentration in Control rats was (6.0 ± 0.1 mIU/L); Streptozotocin (3.7 ± 0.1 mIU/L); Streptozotocin-Insulin (6.0 ± 0.1 mIU/L); Streptozotocin-Ginger (8.5 ± 0.1 mIU/L); Inuslin resistant (3.8 ± 0.1 mIU/L); Inuslin resistant-Pioglitazone (3.2 ± 0.1 mIU/L); Inuslin resistant-Ginger rats (6.0 ± 0.1 mIU/L). It was significantly lower (P < 0.001) in the Streptozotocin; Inuslin resistant and Inuslin resistant-Pioglitazone groups compared to the Control; Streptozotocin-Insulin; Streptozotocin+ Ginger and Inuslin resistant-+ Ginger groups (Table 1).

Plasma LH concentration in Control (0.73 ± 0.08 IU/L); Streptozotocin (0.17 ± 0.01 IU/L); Streptozotocin-Insulin (0.20 ± 0.01 IU/L); Streptozotocin-Ginger (0.57 ± 0.01 IU/L); Insulin resistant (0.40 ± 0.01 IU/L); Insulin resistant-Pioglitazone rats (0.20 ± 0.01 IU/L) and Insulin resistant-Ginger rats (0.1 ± 0.01 IU/L). It was significantly lower (P < 0.001) in all the treated rats compared to the Control rats and this reduction was not reversed by exogenous insulin, pioglitazone and ginger administrations (Table 1).

Plasma FSH concentration in Control (0.08 ± 0.01 IU/L); Streptozotocin (0.09 ± 0.01 IU/L); Streptozotocin-Insulin (0.19 ± 0.01 IU/L); Streptozotocin-Ginger (0.11 ± 0.01 IU/L); Insulin resistant (0.08 ± 0.01 IU/L); Insulin resistant-Pioglitazone (0.19 ± 0.01 IU/L); Insulin resistant-Ginger rats (0.06 ± 0.01 IU/L). It was significantly higher (P < 0.001) in the Streptozotocin-Insulin and Insulin resistant-Pioglitazone groups compared to all the other groups (Table 1).

Plasma Testosterone concentration was significantly higher (P < 0.001) in the Insulin resistant (8.8 ± 0.1 nmol/L); Streptozotocin (5.9 ± 0.1 nmol/L) and Insulin resistant-Pioglitazone (5.4 ± 0.1 nmol/L) groups compared to the Control group (4.9 ± 0.09 nmol/L). However, it was significantly lower (P < 0.001) in the Streptozotocin- Ginger (3.7 ± 0.1 nmol/L) and Streptozotocin-Insulin (4.2 ± 0.07 nmol/L) groups compared to the Control group. It was not significantly different in the Insulin resistant-Ginger rats (4.8 ± 0.1 nmol/L) compared to the Control rats (Table 1).

Testicular Testosterone concentration in the Control (11.3 ± 0.2 nmol/L); Streptozotocin (2.3 ± 0.3 nmol/L); Streptozotocin-Insulin (5.9 ± 0.4 nmol/L); Streptozotocin-Ginger (6.4 ± 0.5 nmol/L); Insulin resistant (5.1 ± 0.5 nmol/L) and Insulin resistant-Pioglitazone (5.6 ± 0.6 nmol/L) and Insulin resistant-Ginger rats (6.6 ± 0.3 nmol/L). It was significantly lower (P < 0.001) in all the treated groups compared to the Control group and this decrease was reversed by exogenous insulin, pioglitazone and ginger administrations (Table 2).

On the other hand, Testicular Cholesterol concentration was significantly higher (P < 0.001) in all the treated groups i.e. Streptozotocin (359.0 ± 4.1 mg/dl); Streptozotocin-Insulin (215.7 ± 8.7 mg/dl nmol/L); Streptozotocin-Ginger (211.2 ± 4.7 mg/dl); Insulin resistant (232.0 ± 4.9 mg/dl); Insulin resistant-Pioglitazone (220.7 ± 3.6 mg/dl) and Insulin resistant-Ginger rats (163.3 ± 6.7 mg/dl) compared to the Control rats (132.3 ± 6.2 mg/dl). This increase was also reversed by exogenous insulin, pioglitazone and ginger administrations (Table 2).

Seminal vesicle fructose concentration was significantly higher (P < 0.001) in the Control rats

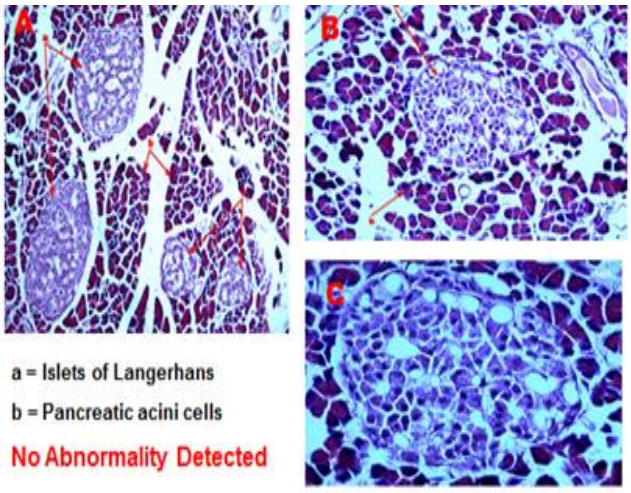

Figure 1: Photomicrograph of control pancreas (A) X100, (B)X200, (C)X400 magnifications

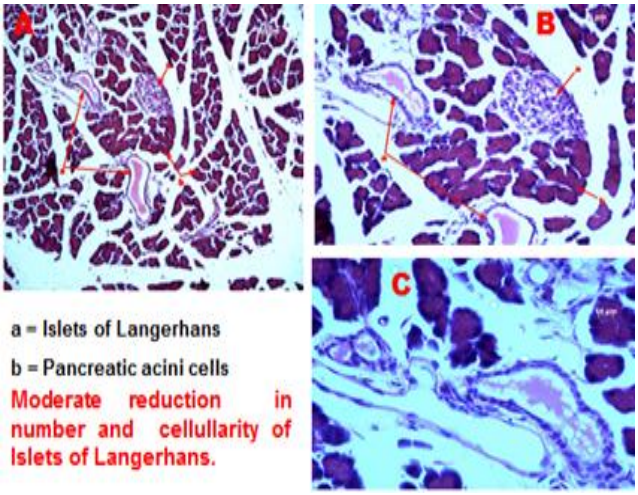

Figure 2: Photomicrograph of Streptozotocin pancreas (A) X100, (B)X200, (C)X400 magnifications

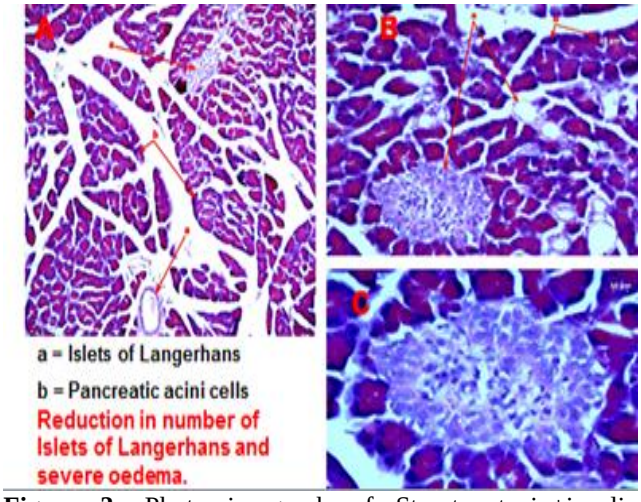

Figure 3: Photomicrograph of Streptozotocin+insulin pancreas (A) X100, (B)X200, (C)X400 magnifications

(4.95 ± 0.46 g/L) compared to the rats in Streptozotocin (1.70 ± 0.30 g/L); Streptozotocin-Insulin (2.00 ± 0.24 g/L) and Streptozotocin-Ginger (2.30 ± 0.24 g/L) groups. As anticipated, seminal vesicle fructose concentration was higher in the

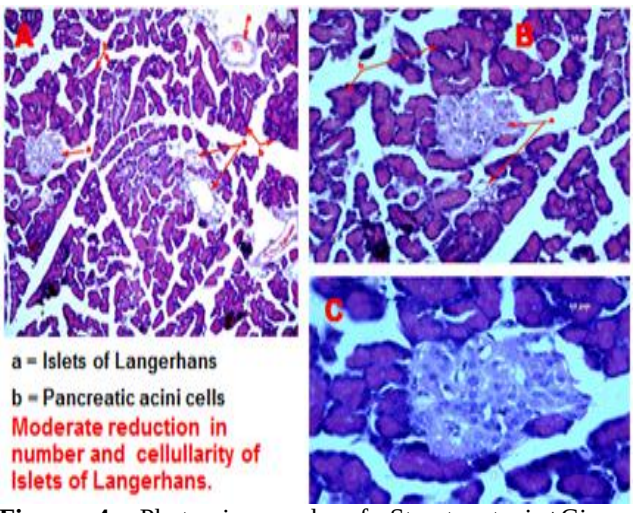

Figure 4: Photomicrograph of Streptozotocin+Ginger pancreas (A) X100, (B)X200, (C)X400 magnifications

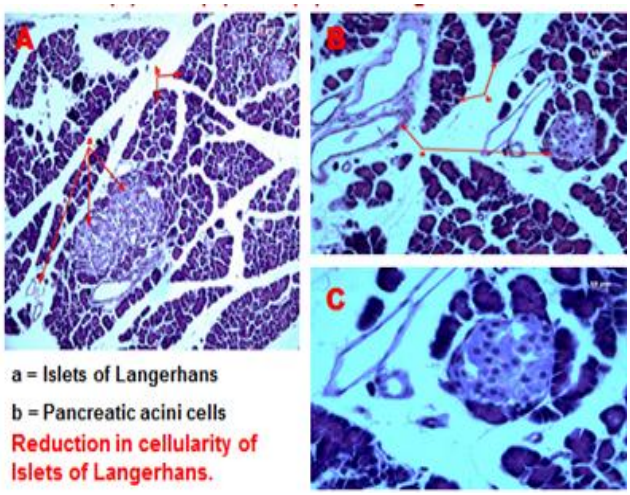

Figure 5: Photomicrograph of Insulin resistant pancreas (A) X100, (B)X200, (C)X400 magnifications

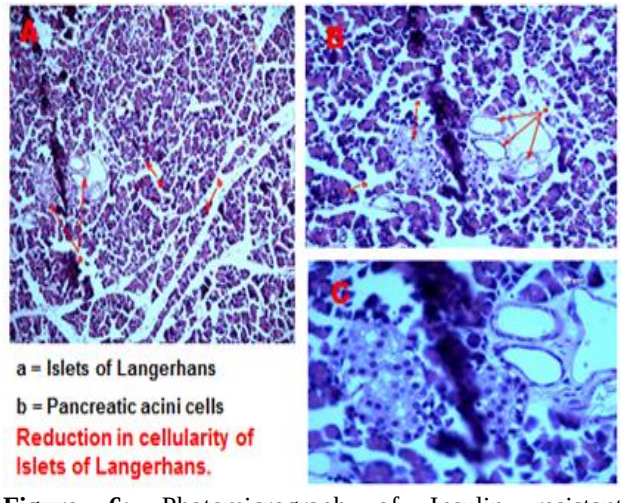

Figure 6: Photomicrograph of Insulin resistant+ Pioglitazone pancreas (A) X100, (B)X200, (C)X400 magnifications

Insulin resistant (5.23 ± 0.47 g/L Insulin resistant-Pioglitazone (5.27 ± 0.38 g/L) and Insulin resistant-Ginger rats (5.67 ± 0.43 g/L) compared to the Control

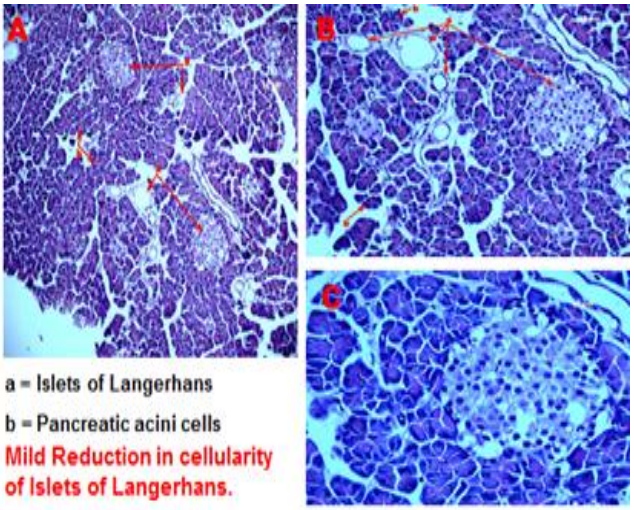

Figure 7: Photomicrograph of Insulin resistant+Ginger pancreas (A) X100, (B)X200, (C)X400 magnifications

rats (4.95 ± 0.46 g/L) but this increase was not statistically significant (Table 2).

Testicular ALP concentration in the Control (36.5 ± 4.8 IU/L); Streptozotocin (129.8 ± 9.9 IU/L); Streptozotocin-Insulin (112.0 ± 7.4 IU/L); Streptozotocin-Ginger (109.0 ± 3.5 IU/L); Insulin resistant (88.0 ± 4.3 IU/L); Insulin resistant-Pioglitazone (75.3 ± 4.2 IU/L) and Insulin resistant-Ginger rats (68.3 ± 4.0 IU/L). It was significantly higher ($P < 0.001$) in all the treated groups compared to rats in the Control group and this increase was slightly reversed by exogenous insulin, pioglitazone and ginger administrations (Table 2).

On the other hand, testicular LDH concentration in the Control rats (156.0 ± 4.2 IU/L); Streptozotocin (81.7 ± 11.9 IU/L); Streptozotocin-Insulin (101.3 ± 7.2 IU/L); Streptozotocin- Ginger (103.0 ± 8.0 IU/L); Insulin resistant (91.7 ± 10.5 IU/L); Insulin resistant-Pioglitazone (104.0 ± 6.0 IU/L) and Insulin resistant-Ginger rats (105.7 ± 7.7 IU/L). This was significantly lower ($P < 0.001$) in all the treated groups compared to rats in the Control group and this decrease was also slightly reversed by exogenous insulin, pioglitazone and ginger administrations (Table 2).

Testicular ACP concentration in Control (0.57 ± 0.07 IU/L); Streptozotocin (0.47 ± 0.07 IU/L); Streptozotocin-Insulin (0.63 ± 0.07 IU/L); Streptozotocin-Ginger (0.63 ± 0.07 IU/L); Insulin resistant (0.52 ± 0.05 IU/L); Insulin resistant-Pioglitazone (0.63 ± 0.05 IU/L) and Insulin resistant-Ginger (0.67 ± 0.05 IU/L). This was not significantly different amongst all the 7 groups (Table 2).

Histopatholgical assessments of the pancreas showed reduction (mild, moderate or severe) in number and cellularity of Islets of Langerhans in all the treated groups compared to the control group. Streptozotocin diabetes and insulin resistance caused varying degrees of pancreatic β cells degranulation.

DISCUSSION

The present results on FBG level support the views that streptozotocin increases blood glucose in rats (Fernandes et al., 2011; Lee et al., 2010) to cause type I diabetes mellitus and that insulin resistance causes type II diabetes mellitus (Arikawe et al., 2006) and that ginger exhibits some hypoglyceamic effects (Iranloye et al., 2011; Al-Amin et al., 2006; Kadnur and Goyal, 2005; Akhani et al., 2004). The present result on glycosylated haemoglobin level is also in line with the views of Bonnefont-Rousselot et al., (2000) and Punithavathi et al., (2011). The effects of streptozotocin and insulin resistance on both FBG and $HbA1_c$ were reversed by insulin, pioglitazone and ginger administrations.

As expected, streptozotocin and insulin resistance increased both the FBG and $HBA1_c$ levels while significantly decreasing ($P < 0.05$) plasma insulin levels. This was also reversed by insulin, pioglitazone and ginger administrations. Streptozocin and chronic fructose consumption caused structural alterations in pancreatic β cells (Lee at al., 2010; Van Assche et al., 1983) to cause hypoinsulinaemia (Streptozotocin group) and hyperinsulinaemia (insulin resistant group). This was observed in all the photomicrographs on pancreas of the treated groups. This effect was reversed by insulin, pioglitazone and ginger with a corresponding increase in plasma insulin levels. This further confirms that exogenous insulin, pioglitazone and ginger are potent insulin sensitizers (Triplitt et al., 2010) i.e. effective in increasing plasma insulin levels and also augment β cell function (Gastaldelli et al., 2007).

The results on plasma LH and FSH concentrations could be explained based on the various interplay that exist within the hypothalamo-pituitary-testicular axis i.e. LH secretion is under negative feedback mechanism which implies that testicular steroids act predominantly within the CNS to suppress GnRH secretion (Tilbrook and Clarke, 2001) while FSH secretion on the other hand, is under negative feedback mechanism with Inhibin-B acting directly at the pituitary gland (Tilbrook and Clarke, 2001). However, Inhibin-B was not measured during the course of this study and will be a subject of further research in these groups of animals.

The results on LH is in line with the view of Fernandes et al., (2011). Comparing this with that of plasma FSH and plasma testosterone, it appears that the hypothalamo-pituitary-testicular axis is dysfunctional in the treated groups and this dysfunction was ameliorated to some extent by exogenous insulin, pioglitazone and ginger administration. This point could also be confirmed based on the histology of the pituitary gland in the treated groups (unpublished data).

Prolactin concentration was significantly lower ($P < 0.001$) in the Streptozotocin only; Fructose only

and Fructose + Pioglitazone groups compared to the other groups. This is in line with the view of Sudha et al., (2000), who reported that prolactin concentration is significantly reduced in diabetic male rats .

Testosterone is essential for spermatogenesis completion because it stimulates the conversion of round spermatids into elongated spermatids between stages VII and VIII of the spermatogenic cycle (Hammami et al., 2008). Thus, testicular testosterone deficiency as observed in this study will impair the spermiation process (Saito et al., 2000). This further supports the view that diabetes mellitus causes a defect in spermiogenesis (Shetty et al., 1998). Likewise, decreased testicular testosterone has been previously associated with histological alterations in androgen target cells i.e. Leydig and Sertoli cells (Yang et al., 2006).

Seminal fructose concentration was significantly lower ($P < 0.001$) in all the treated groups compared to the rats in the Control group, with the exception of all the insulin resistant groups. This was expected since fructose was chronically fed to these groups of animals. Fructose provides energy for sperm motility (Elzanaty et al., 2002; Williams and Ford, 2001). However, despite a high seminal fructose level in the insulin resistant groups compared to control and streptozotocin groups, percentage sperm motility was still significantly lower in diabetic and insulin resistant rats (Arikawe et al., 2006). This fact could be a basis for future research on the relationship between sperm nuclear DNA damage and sperm motility in diabetic and insulin resistant male rats.

The two key enzymes ALP and LDH were altered in the diabetic and insulin resistant animals compared to the control animals. This is in line with the views of the following authors Prakash et al., (2011); Prasath and Subramanian, (2011); Yamaguchi et al., (2007) and Hough et al., (1981) who have all reported that ALP and LDH levels are altered in diabetic conditions. On the other hand, testicular ACP concentration was not significantly different amongst all the 7 groups. This could be because it is widely distributed in lysosomes of spermatogonia, Sertoli cells and spermatids (Chemes, 1986).

The results of this study showed that both central and local mechanisms are implicated in the impairment of spermatogenesis by diabetes but the hypothalamo-pituitary testicular axis alteration might not likely have a major impact as the local defect on steroidogenesis in the testis. This local defect could also predispose to male hypogonadism, i.e. failure of gonadal function.

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