Insulin, pioglitazone and *Zingiber officinale* administrations improve proliferating cell nuclear antigen immunostaining effects on diabetic and insulin resistant rat testis

Adesina Paul Arikawe*, Adetola Olubunmi Daramola*, Ibiyemi Ibilita Olutunji-Bello*, Leonard Fidelis Obika*

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**ABSTRACT**

This study accessed the effects of hypoglycaemic drugs on spermatogenesis in diabetic and insulin resistant rat testis following proliferating cell nuclear antigen (PCNA) immunostaining. Male adult Sprague-Dawley rats (120-140 g) were randomly divided into 5 groups. Group 1 served as control group; fed on normal rat pellets. Group 2 served as streptozotocin-insulin treated group; received a single dose IP Injection of streptozotocin 45 mg/kg BW in Na+ citrate buffer pH 4.5 and treated with insulin sub-cutaneously. Group 3 served as 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 4 served as Insulin resistant-pioglitazone treated group; fed ad libitum on a special diet containing 25% fructose mixed with 75% normal rat chow (w/w) and treated with Pioglitazone 15 mg/kg orally. Group 5 served as 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. Following hyperglycemia confirmation, animals were perfused with 4% Paraformaldehyde (PFA). Testes were isolated, and fixed in 4% PFA overnight, embedded in paraffin, 5µm thick sections were made and mounted on poly-L-lysine coated slides. Immunohistochemical staining was carried out on positively charged slides using PCNA as primary antibody. The results showed that insulin, pioglitazone and ginger administrations ameliorated the diabetic and insulin resistant effects on spermatogenesis in male rats. The precise interplay of hypothalamic, pituitary and steroid hormones is subject for further study in these group of animals.

**Keywords:**

Diabetes
Ginger
Insulin
PCNA
Pioglitazone
Spermatogenesis

1. Introduction

The testis is a tissue of high proliferative activity in which, sperm cells (spermatozoa) are produced from stem cells (spermatogonia) by two consecutive steps of cell multiplication and spermatid cytodifferentiation (Stephan et al., 1996). Diabetes mellitus is a common health problem with many functional and structural complications (Todd Cade, 2008). The World Health Organization (WHO) in year 2000 reported that 177 million people were affected by diabetes worldwide and projected that by 2025, this figure will rise to over 300 million (WHO, 2002). Likewise, Zimmet et al., (2001) also projected that this figure will be doubled in the next 20 years.

Insulin is the major hormone controlling critical energy functions such as glucose and lipid metabolism. Colosia et al., (1988) in their work showed that when diabetic rats were treated with insulin, the activities of the bifunctional enzyme 6-phosphofructo-2-kinase/fructo-2,6-diphosphate were restored back to normal after falling to 20% of Control values and this fall was associated with insulin deficiency (Dall’Aglion et al., 1986). Pioglitazone is a potent insulin sensitizer, which binds to the peroxisome-proliferator activated receptor-gamma, resulting in enhanced muscle, liver, and adipose tissue sensitivity to insulin, with a resultant decline in fasting and postprandial plasma glucose levels (Miyazaki et al., 2002; Matthews et al., 2005; Miyazaki and De Fronzo, 2008). Pioglitazone has also been reported to augment beta cell function (Gastaldelli et al., 2007).
Ginger (Zingiber officinale) is a commonly used spice in the African Kitchen and has been used as spice for over 2000 years (Bartley and Jacobs, 2000). It is cultivated in the tropics for its edible rhizome at about 10 months of age, with the root stocks serving culinary and medicinal purposes (Portnoi et al., 2003). The culinary use is as spice and food ingredient while for its medicinal use, research on rats suggests that ginger may be useful for treating diabetes (Kim et al., 2003). Al-Amin et al., (2006) observed that aqueous ginger juice has anti-diabetic and hypolipidaemic properties. Akhani et al. (2004) also observed that ginger juice exhibits hypoglycemic activity in both normal and Type 1 diabetic rats.

Proliferating Cell Nuclear Antigen (PCNA) is a 36kDa non-histone protein found in the nucleus that plays a role in the initiation of cell proliferation by mediating DNA polymerase, which plays an integral role in the eukaryotic cell cycle and is essential for cellular DNA synthesis. PCNA is a primary antibody; its levels are elevated in the S, G2, and M phases of cell mitosis in normal and malignant tissues. Its biological half-life is 20 hours and its expression has a broad correlation with mitotic activity and can be used as a marker for cell proliferation (Bravo and Macdonald-Bravo, 1987, Waseem and Lane, 1990; van Dierendonck et al., 1991; Dervan et al., 1992; Linden et al., 1992). PCNA is useful for the diagnosis of germinal arrest because there are significantly reduced PCNA levels in germinal arrest, which is an indication of DNA synthesis deterioration (Zeng et al., 2001).

Diabetes is also caused by complicated factors e.g. absolute insulin deficiency and relative insulin deficiency i.e. insulin resistance and its damage to the male reproductive system has been reported to threaten men’s health (Zhao et al., 2004). Male sexual and reproductive functions are one of the mammalian systems that are clearly impaired by Diabetes (Guneli et al., 2008; Jelodar et al., 2010). In line with this view, Arikawe et al., (2006) reported that diabetes mellitus and insulin resistance affect semen parameters and impair spermatogenesis in male rats. Furthermore these same authors recently reported that streptozotocin-induced diabetes and insulin resistance reduced PCNA index, mean seminiferous tubular diameter and testicular diameter in adult male rats (Arikawe et al., 2012). Thus, the present study aimed to assess PCNA immunostaining effects in streptozotocin-induced and insulin resistant diabetic rat testis after treatment with insulin, pioglitazone and Zingiber officinale.

2. Experimental procedure
Thirty-five 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 26°C-28°C 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 5 groups (of 7 animals each of similar weight).

Group 1 served as Control group, fed on normal rat chow throughout experimental period of 16 weeks. Group 2 served as Streptozotocin-insulin treated group; fed on normal rat chow; 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, and also treated with 0.5-1 IU Isophane Insulin sub-cutaneously for additional 4 weeks. Group 3 served as Streptozotocin-ginger treated group; fed on normal rat chow; received Streptozotocin injection as in Group 2 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; Alnaqeeb et al., 2003; Morakinyo et al., 2008). Blood samples were collected from the tail vein 48 hours after Streptozotocin injection to confirm hyperglycemia 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 glycemic levels were very high, and as time passed the animals became weaker (Watala et al., 2009) and eventually resulted in mortality.

Group 4 served as Insulin resistant-pioglitazone treated 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 12th week (Arikawe et al., 2006); and also treated with Pioglitazone 15mg/kg orally for additional 4 weeks. Group 5 served as Insulin resistant-ginger treated group; fed ad libitum on a special diet as in group 4 above, and also treated with 500mg daily of ginger extract/Kg body weight orally for additional 4 weeks. Hyperglycemia was confirmed at the 16th 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 5). 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 Type I 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) Immunohistochemical staining using PCNA as primary antibody.

Immunohistochemical staining
At the end of each experimental period, following hyperglycaemia confirmation, animals were perfused using standard Perfusion Techniques in the laboratory with 4% Formaldehyde (PFA), this was carried out at high stringency. Summarily before removal of the testes, the rats were deeply
anaesthetized with diethly ether and pain suppressed using sodium pentobarbitone. Rats were then perfused transcardially first with Dulbecco Phosphate Buffer Saline (PBS) and followed by 4% PFA in PBS. Successful perfusion was confirmed by spontaneous movement (formalin dance), rapid tail rise, stretching, lightened colour of liver and pale testis. Testes were isolated, weighed and fixed in 4% PFA overnight; they were passed through ascending series of ethanol baths, embedded in paraffin, 5µm thick sections of paraffin-embedded samples were made using the microtome machine and mounted on poly-L-lysine coated slides i.e. positively charged slides. Routine deparaffinization and rehydration steps were carried out, followed by antigen retrieval steps (ARs). Antigen retrieval (or antigen recovery) is performed to expose or retrieve antigens which have become masked by the tissue fixation process.

Following successful antigen retrieval steps, immunohistochemical staining was carried out on the positively charged slides using the PCNA as primary antibody. The slides were incubated in 1% Bovine Serum Albumin in PBS (blocking solution) for 30 minutes at room temperature and washed three times in PBS; slides were incubated in pre-diluted primary antibody (PCNA, Invitrogen USA) for 60 minutes at room temperature in a humidified chamber and then washed with PBS twice for 5 minutes each.

Further immunostaining was performed using labeled Streptavidin Horse Radish Peroxidase (HRP) for another 60 minutes at room temperature in a humidified chamber and then washed with PBS twice for 5 minutes each; final step of antigen localization of peroxidase deposition was achieved by using Diaminobenzidine-H₂O₂ (DAB, Dako) for chromogenic reaction and subsequently washed in running water for another 5 minutes; this was followed by counterstaining with Harris’ Haematoxylin and Eosin, dehydrated in alcohols to xylene and finally mounted using Dibutylpthalate Xylene (DPX) with coverslip. As a control for method specificity, in negative control slide, the step using primary antibody (PCNA) was omitted and slides were viewed under the light microscope.

Ten seminiferous tubules were counted in each slide; cells with brown nuclear staining were considered positive (Altay et al., 2003). Both stained and non-stained germ cells were counted and the ratio of stained cells to the total number germ cells, i.e. PCNA index and density was calculated (Altay et al., 2003). The average PCNA index in each case was obtained by dividing the sum of all PCNA indices by the number of seminiferous tubules in which the calculation was carried out. Testicular diameter (TD) was measured directly by cutting testes vertically into two and largest TD then measured (Altay et al., 2003). Mean Seminiferous Tubule Diameter (MSTD) was evaluated by measuring the smallest diameter of ten tubuli on ten randomly selected fields using an ocular micrometer at x400 magnification (Altay et al., 2003).

### 3. Results

Fasting blood glucose concentration (mg/dl) and Glycosylated haemoglobin level (%) in control rats were (91.4±2.1 mg/dl, 5.5±0.2%); Streptozotocin-insulin treated (283.4±13.1mg/dl, 6.6±0.1%); and Streptozotocin-ginger treated (423.3±18.6mg/dl, 6.9±0.2%); Insulin resistant-pioglitazone treated (101.5±2.3mg/dl, 7.8±0.3%); and 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).

<table>
<thead>
<tr>
<th>Group</th>
<th>FBG mg/dl</th>
<th>HbA1c (%)</th>
<th>MSTD (µm) Left Testis</th>
<th>MSTD (µm) Right Testis</th>
<th>MSTD (µm) Both Testes</th>
<th>Testicular Diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>91.4±2.1</td>
<td>5.5±0.2</td>
<td>201.5±6.4</td>
<td>219.8±9.4</td>
<td>210.6±6.1</td>
<td>15.7±0.4</td>
</tr>
<tr>
<td>Streptozotocin-Insulin</td>
<td>283.4±13.1†‡</td>
<td>6.6±0.1†</td>
<td>160.0±7.6a</td>
<td>160.6±7.6†‡</td>
<td>160.1±5.2†‡</td>
<td>13.2±0.5†‡</td>
</tr>
<tr>
<td>Streptozotocin-Ginger</td>
<td>423.3±18.6†‡#</td>
<td>6.9±0.2†</td>
<td>175.4±10.1</td>
<td>167.1±9.9†</td>
<td>175.4±7.6†</td>
<td>13.5±0.6†‡</td>
</tr>
<tr>
<td>Insulin resistant-Pioglitazone</td>
<td>101.5±2.3</td>
<td>7.8±0.3‡*</td>
<td>150.0±6.9†</td>
<td>150.1±7.0†‡</td>
<td>150.0±4.7†‡</td>
<td>11.8±0.6†‡</td>
</tr>
<tr>
<td>Insulin resistant-Ginger</td>
<td>117.1±2.5</td>
<td>7.8±0.1†*</td>
<td></td>
<td></td>
<td></td>
<td>12.8±0.6†‡</td>
</tr>
</tbody>
</table>

*All results presented in mean±SEM
†P<0.001; ‡P<0.01; aP<0.05 Vs Control; #P<0.001 Vs Streptozotocin-Insulin; *P<0.001 Vs Streptozotocin-Ginger;
ªP<0.001 Vs Insulin resistant-Ginger; ¶P<0.001 Vs Insulin resistant-Pioglitazone
Footnote: Mean Seminiferous Tubule Diameter (MSTD); Testicular Diameter (TD); Glycosylated Haemoglobin level (HbA1c (%)

### Table 1. Fasting Blood Glucose concentration, Glycosylated Haemoglobin level, MSTD, and TD in Control, Streptozotocin-Insulin, Streptozotocin-Ginger, Insulin resistant-Pioglitazone, and Insulin resistant-Ginger groups

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. The image I software package was used to analyze the immunohistochemical slides.

### Footnote

- Mean Seminiferous Tubule Diameter (MSTD); Testicular Diameter (TD); Glycosylated Haemoglobin level (HbA1c (%)
- All results presented in mean±SEM
- †P<0.001; ‡P<0.01; aP<0.05 Vs Control; #P<0.001 Vs Streptozotocin-Insulin; *P<0.001 Vs Streptozotocin-Ginger;
- ‭ªP<0.001 Vs Insulin resistant-Ginger; ¶P<0.001 Vs Insulin resistant-Pioglitazone

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0.4 mm); Streptozotocin-insulin treated rats (13.2±0.5 mm); Streptozotocin-ginger treated rats (13.5±0.6 mm); Insulin resistant-pioglitazone treated rats (11.8±0.6 mm) and Insulin resistant-ginger treated rats (12.8±0.6 mm). Testicular diameter was significantly lower (p<0.001) in all the experimental groups compared to rats in the Control group (Table 1).

PCNA Index (%) in the left testis was significantly lower (p<0.001) in the Streptozotocin-insulin treated (64.5±0.2%); Streptozotocin-ginger treated (65.4±0.1%); Insulin resistant-pioglitazone treated (67.3±0.2%); and Insulin resistant-ginger treated rats (43.5±0.2%) compared to rats in the Control (82.8±0.1%) group. In the right Testis, PCNA was also significantly lower (p<0.001) in the Streptozotocin-insulin treated (64.8±0.1%); Streptozotocin-ginger treated (64.5±0.1%); Insulin resistant-pioglitazone treated (67.2±0.1%); and Insulin resistant-ginger treated rats (34.9±0.3%) compared to the control rats (88.4±0.1%) group. Likewise, PCNA index in both testes was significantly lower (p<0.001) in the Streptozotocin-insulin treated rats (64.8±0.1%); Streptozotocin-ginger treated (64.5±0.1%); Insulin resistant-pioglitazone treated (67.2±0.1%); and Insulin resistant-ginger treated rats (34.9±0.3%) compared to the control rats (85.5±0.1%) (Table 2).

Numerical Density (ND) of Seminiferous tubule (µm²) of the left testis in Control rats was (0.04±0.005 µm²); Streptozotocin-insulin treated rats (0.02±0.002 µm²); Streptozotocin-ginger treated rats (0.02±0.002 µm²); Insulin resistant-pioglitazone treated rats (0.04±0.002 µm²); and Insulin resistant-ginger treated rats (0.04±0.005 µm²). Thus, ND of the left testis was significantly lower (p<0.001) in Streptozotocin-insulin and Streptozotocin-ginger treated rats compared to the rats in Control group while there was no significant difference between the control rats and Insulin resistant-pioglitazone and Insulin resistant-ginger treated rats.

On the other hand, ND of the right testis was significantly lower (p<0.001) in the Streptozotocin-insulin treated rats (0.02±0.003 µm²); Streptozotocin-ginger treated rats (0.03±0.002 µm²); Insulin resistant-pioglitazone treated rats (0.03±0.002 µm²) and Insulin resistant-ginger treated rats (0.03±0.003 µm²) compared to the control rats (0.2±0.02 µm²). Likewise, ND of both testes was significantly lower (p<0.01) in the Streptozotocin-insulin treated rats (0.02±0.003 µm²); Streptozotocin-ginger treated rats (0.03±0.002 µm²); Insulin resistant-pioglitazone treated rats (0.04±0.002 µm²) and Insulin resistant-ginger treated rats (0.03±0.005 µm²) compared to the control rats (0.14±0.05 µm²) (Table 2).

The immunohistochemical staining of seminiferous tubules in all the groups showed that positive slides for all the groups were reactive i.e. nuclei of the cells were brown coloured stained. Thus are positive for PCNA staining. In Figure 1, all the cell lines were present and no abnormality was detected. In Figure 2, secondary spermatocytes and elongated spermatids were not identified. In addition to this, there was also global necrosis and mild reduction in number of germ cells. In Figure 3, older spermatids were not identified. In addition to this, there was mild reduction in number of germ cells and spermatids. In Figure 4, primary spermatocytes, secondary spermatocytes and older spermatids were not identified. In addition to this, there was also focal necrosis and mild reduction in number of germ cells. In Figure 5, secondary spermatocytes, older and elongated spermatids were not identified. In addition to this, there was also reduction in number of spermatids.

Table 2. PCNA index and Numerical density in Control, Streptozotocin-Insulin, Streptozotocin-Ginger, Insulin resistant-Pioglitazone, and Insulin resistant-Ginger groups

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Streptozotocin-Insulin</th>
<th>Streptozotocin-Ginger</th>
<th>Insulin resistant-Pioglitazone</th>
<th>Insulin resistant-Ginger</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PCNA Index (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Left Testis</strong></td>
<td>82.8±0.1</td>
<td>64.5±0.2†</td>
<td>65.4±0.1#</td>
<td>67.3±0.2##</td>
<td>34.5±0.2##*†</td>
</tr>
<tr>
<td><strong>Right Testis</strong></td>
<td>88.4±0.1</td>
<td>64.8±0.1†</td>
<td>65.4±0.1†</td>
<td>67.2±0.1##</td>
<td>34.9±0.3##*†</td>
</tr>
<tr>
<td><strong>Both Testes</strong></td>
<td>85.2±1.2</td>
<td>64.8±0.1†</td>
<td>64.9±0.3†</td>
<td>67.1±0.1†</td>
<td>34.6±0.2##*†</td>
</tr>
<tr>
<td><strong>Numerical Density</strong></td>
<td>0.04±0.005</td>
<td>0.02±0.002†</td>
<td>0.02±0.002†</td>
<td>0.04±0.002η</td>
<td>0.04±0.005η</td>
</tr>
<tr>
<td><strong>Both Testes</strong></td>
<td>0.14±0.05</td>
<td>0.02±0.003†</td>
<td>0.03±0.002†</td>
<td>0.04±0.002†</td>
<td>0.03±0.003†</td>
</tr>
</tbody>
</table>

All results presented in mean ± SEM
†p<0.001; ¶p<0.01 Vs Control; #p<0.001; ηp<0.01 Vs Streptozotocin-Insulin; *p<0.001 Vs Streptozotocin-Ginger; **p<0.01 Vs Insulin resistant-Ginger
4. Discussion

Fasting blood glucose concentration (FBG) and Glycosylated haemoglobin level (HbA1c) were significantly higher (p<0.001) in all the experimental groups compared to rats in the Control group, with the exception in the Insulin resistant-pioglitazone treated and Insulin resistant-ginger treated rats, were FBG was not significantly different compared to that of the control rats.

The results on FBG level support the views that streptozotocin increases blood glucose in rats (Altay et al., 2003; Gunelli et al., 2008; Lee et al., 2010; Fernandes et al., 2011) to cause type I diabetes mellitus and that chronic fructose consumption through insulin resistance mechanism causes type II diabetes mellitus (Arikawe et al., 2006). The results also show that ginger exhibits some hypoglycaemic effects (Akhani et al., 2004; Kadnur and Goyal, 2005; Al-Amin et al., 2006; Iranloye et al., 2011). The glycaemic level was well controlled in the control animals (HbA1c<6.5%) while in all the experimental groups, it was not well controlled (HbA1c>6.5%) (Bonnefont-Rousselot et al., 2000; Punithavathi et al., 2011). The results on both FBG and HbA1c were ameliorated by insulin, pioglitazone and ginger administrations.

PCNA reacts intensely with the nuclei of cells at the pre-leptotene, leptotene through pachytene stages of spermatogenesis but it does not react with the nuclei of Sertoli cells (Wrobel et al., 1996). This was observed in the positive slides (with primary antibody) for all the groups which were reactive i.e. nuclei of the cells were brown colour stained. Thus, were termed positive for PCNA (Altay et al., 2003). Furthermore, there was necrosis (focal or global) and reduction (mild or moderate) in germ (viable) cells and spermatids in both treated groups compared to the Control group.

MSTD of both testes was significantly lower (p<0.001) in all the experimental groups compared to the Control group. This effect progressed with the duration of the diabetic state and appears to be ameliorated more by ginger administration. This is in line with the view of Fernandes (Fernandes et al., 2011) who reported that vitamin C (an anti-oxidant like ginger) restored seminiferous tubular diameter in streptozotocin-induced diabetic male rats.

TD, PCNA index (%), and ND were also significantly reduced (P<0.001) in all the rats in the experimental groups compared to the rats in the Control group. This is in line with the view of Altay et al. (2003) and Jelodar et al. (2010). The results confirm that diabetes mellitus and insulin resistance impair spermatogenesis in male rats (Arikawe et al., 2006) and that spermatogenic cell cycle series were decreased in the streptozotocin diabetic and insulin resistant diabetic groups (Arikawe et al., 2012).

Treatment with insulin, pioglitazone and ginger ameliorated some of the effects of diabetes mellitus and insulin resistance on MSTD, PCNA, ND and TD. However, these reversed levels were still significantly lower than the Control levels. This could be because insulin, pioglitazone and ginger are insulin sensitizers (Triplitt et al., 2010) and also because diabetes related testicular effects have been attributed to the lack of insulin (Jelodar et al., 2010).

Conclusion

The results of this study showed that insulin, pioglitazone and ginger administrations ameliorated the diabetic and insulin resistant effects on spermatogenesis in male rats. The precise interplay of hypotalamic, pituitary and steroid hormones is subject for further study in this group of animals.

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