Effect of Obestatin on Testicular Functions of Normal and Streptozotocin Induced Type 1 Diabetic Rats

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Abstract

Background: Diabetes Mellitus (DM) is a metabolic disease induces serious complications related to its oxidative stress state. Obestatin is a peptide hormone produced by different tissues and expressed with its receptors in testes and it has been reported to have antioxidant properties.

Objective: This work was designed to investigate the effects of diabetes mellitus on testicular functions and the role of obestatin in modulating these effects in Streptozotocin (STZ) induced rat model of type 1 diabetes mellitus.

Material and Methods: Experiments conducted on 32 healthy adult male albino rats (193 ± 9.14gm), which were randomly and equally divided into 2 groups, Group I (normal) and Group II (STZ “60mg/kg” induced diabetic group). Each group further subdivided into equal 2 subgroups: Group A (received a single ip injection of 100 µl saline daily for 30 consecutive days) and Group B (daily ip injected with obestatin “1nmol/100gm BW” for 30 consecutive days). Serum levels of glucose, insulin, FSH, LH and testosterone levels were measured. Epididymal sperm count, motility, and percentage of deformity were detected; testes weight, testicular malondialdehyde (MDA) level and Superoxide Dismutase (SOD), Catalase (CAT) and Glutathione Peroxidase (GPX) activities were estimated plus testicular histopathology.

Results: STZ-induced diabetes significantly decreased testes weight, serum insulin, FSH, LH and testosterone levels, in addition to a significant reduction in epididymal sperm count, motility and testicular SOD, CAT and GPX activities, plus significantly increased serum glucose, percentage of deformed sperm and testicular MDA levels together with deterioration of the testicular histoarchitecture. Moreover, it was found that chronic daily administration of obestatin resulted in a significant recovery of all the deteriorated testicular parameters in the diabetic group, while in the normal group, it was only associated with potentiation of the testicular antioxidant system as well as a significant increase in testes weights and sperm count.

Conclusion: Obestatin, being owns antioxidant properties and can maintain normal levels of glucose and insulin homeostasis, it has a protective role against diabetes-induced testicular dysfunction. Chronic obestatin administration can improve metabolic state and guard against diabetes induced hypofertility.

Key Words: Obestatin – Diabetes – Testicular function – Oxidative stress.

Introduction

OXIDATIVE Stress (OS) is an imbalance between the production of Reactive Oxygen Species (ROS) and the ability of biological systems to readily detoxify the reactive intermediates or to repair the resulting damage of cell biomolecules, such as nucleic acids, proteins, structural carbohydrates, and lipids [1].

OS is a major factor in the etiology of male infertility; independent clinical studies have demonstrated a correlative relationship between male infertility and evidence of OS in the ejaculate [2,3]. At the level of the isolated spermatozoon, ROS attack can induce lipid peroxidation and DNA fragmentation disrupting both the motility of these cells and their ability to support normal embryonic development [4,5]. So, antioxidants may be useful in the treatment of male infertility [6,7].

Diabetes Mellitus (DM) is a global metabolic disorder characterized by hyperglycemia with insufficient insulin production; DM type 1 (DM 1) or action DM type 2 (DM2) [8]. Long term hyperglycemia plus the OS state of DM play great role in pathogenesis of diabetes and lead to high risk of macro-and micro-vascular complications [9]. Moreover, the balance between oxidant and antioxidant species has been proposed to have an important role in preventing diabetic complications [6].

Cases of infertility are increasing among diabetic males [10]. Regarding the impact of DM on
testicular function, while diabetic men may represent normal semen parameters [11], they have a higher level of damage in sperm nuclear and mitochondrial DNAs [12], that may lead to impairment in the percentage of spermatozoa with progressive motility reduction especially with uncontrolled DM 1 [13]. In contrast, other investigators reported unaffected pituitary gonadotrophins and testosterone production [14] and higher sperm were gathered with no differences to take place in motility in DM1 patients [15].

Obestatin is a 23 amino acids peptide derived from the same precursor protein as ghrelin and is the cogent ligand for GPR39, but its biological activity is reported to be opposite to that of ghrelin, as obestatin suppresses food intake and body weight gain in mice and rats [16-18]. Although the majority of obestatin is produced by the stomach, the obestatin peptide has been reported to be expressed in a range of peripheral tissues, for example the pancreas [19,20], thyroid [21], gastrointestinal tract [22], and testis [23]. This may indicate that obestatin has local autocrine/paracrine roles in addition to its actions as an endocrine hormone.

Obestatin expression has been proved in the Leydig cells of testes [23], in addition obestatin had a stimulatory role in the modulation of cellular proliferation during the peripubertal period in male rats, and single i.v injection of obestatin increased testosterone secretion in adult male rats [24,25]. However, the specific role of obestatin in the regulation of testicular functions is still unclear.

The present study aimed to investigate the effect of Streptozotocin (STZ) induced DM1 on testicular functions and role of chronic obestatin administration on testicular functions in normal and STZ induced diabetic rat model.

Material and Methods

32 experiments done using healthy adult male albino rats (193±9.14gm), were purchased from the Animal House of Faculty of Veterinary Medicine-Zagazig University. Rats were kept in the Physiology Animal House in Faculty of Medicine-Zagazig University. All animals received care in compliance with the animal care guidelines and ethical regulations in accordance with the guide for the care and use of laboratory animals according to Institute of Laboratory Animal Resources, [26], the animals were housed in plastic cages under controlled hygienic conditions with an ambient temperature (22±2°C) and a 12 hours light-cycle. They fed standard rodent food and had free access to water. All animals were subjected to 7 days period of passive preliminaries for adaptation.

In the period from 4th February to 23th of April 2016, rats were randomly divided into two equal mean groups, Group I: Normal (non-diabetic) rats and Group II: Streptozotocin (STZ) induced diabetic group, in which experimental diabetes was induced by an i.p injection of freshly prepared streptozotocin (Sigma Aldrich Co.-USA) at a dose of 60mg/kg dissolved in saline [27], in overnight fast rats. After 72 hours, fasting glucose level was measured using glucometer (ACCU CHEK, Rhoche Diagnostics, Germany) and rats with blood glucose levels above 360mg/dl were included in this study [14] with weekly monitoring of those levels throughout the study.

Experimental grouping: Each of Group I (normal) and Group II (diabetic) divided into equal two subgroups (n=8). Subgroup (A) (vehicle treated) group, each rat received a single ip injection of 100 µL saline "NaCl 0.9%" daily for 30 consecutive days, subgroup (B): Obestatin treated, rat received a single ip daily dose of obestatin (1nmol/100gm BW dissolved in 100 µL saline) (Sigma Aldrich Co.-USA) for 30 consecutive days [25].

Sample collection:

24 hours after the last injection of obestatin, blood samples were collected from retro-orbital venous plexus and serum was separated by centrifugation of clotted blood at 3000rpm for 20 minutes. The serum was kept deep frozen at (−80°C) until it was analyzed. After collecting blood samples, laparotomy was conducted after the animals were sacrificed by cervical dislocation under mild ether anesthesia. Testes and epididymis were collected. The epididymis was used for the evaluation of sperm parameters. The testes were weighed then the right one was prepared for histopathological study and the left one was homogenated for biochemical analysis of MDA levels and SOD, GPX and CAT activities.

Analysis of sperm parameters:

The epididymis of each rat was dissected, removed and incubation in 2ml of Hank’s Buffer Salt Solution (HBSS) at 37°C [14]. 5 minutes later, the caudal epididymis sperm was determined using the standard hemocytometer slide (HBG Company, Giessen, Germany) in white blood cell chambers by light microscopy (Olympus Light Microscope, Tokyo, Japan). Finally, data were expressed as the count of sperm per milliliter [28]. The percentage
of sperm motility was calculated using the number of live sperm cells over the total number of sperm cells, and the percentage of deformed sperm was also calculated [29].

Biochemical analysis:

Serum was analyzed for: Glucose levels according to Tietz, [30] using glucose enzymatic (GOD- PAP)-liquizyme Kits (Biotechnology, Egypt), insulin levels according to Temple et al., [31] using KAP 125 1-INS-EASIA (Enzyme Amplified Sensitivity Immunoassay) kits (BioSource Europe S.A., Belgium), LH, FSH and testosterone levels were detected according to Tietz. [30] using ELISA rat kits: BC-1031, BC-1029, and BC-1115, respectively, Bio Check Inc 323 Vintage Park Dr. Foster City.

Preparation of testis homogenate:

The left testes were sliced and homogenized in 50mM of cold phosphate buffer (pH 7.0) containing EDTA (0.1mM) to give 10% homogenate (w/v). The homogenates were centrifuged at 1000r.p.m. for 10min [32]. The supernatants were separated and used for antioxidant system evaluation.

Testicular antioxidant system evaluation:

Assay of Superoxide Dismutase (SOD) activity: According to the method of Kakkar et al., [33], Catalase (CAT) activity: According to the method of Luck [34], Glutathione Peroxidase (GPX) activity: According to the method of Reddy et al., [35]. Assay of MDA level according to the method of Ohkawa et al., [36]. All are measured by using spectrophotometer (spectronic 3000 Array, Germany) at 560, 240, 430, 535nm respectively.

Histopathological examination of right testes:

Tunica vaginalis was carefully removed and the testis were dissected out and cleaned with cold physiological saline to remove blood and the adhering tissues. The samples were then fixed in 10% formaldehyde in fresh alcoholic Bouin’s fluid for 8 hours, and then processed and embedded in paraffin wax, sectioned at 5 µm thickness, and stained in hematoxylin-eosin [37].

Statistical analysis:

The data were expressed as mean ± SD for quantitative variables and statistically analyzed by using SPSS program (Version 22 for windows) (SPSS Inc. Chicago, IL, USA). One way analysis of variance (ANOVA) was used to compare the results of all examined groups followed by LSD test to compare statistical differences between groups. p-value <0.05 was considered statistically significant.

Results

Table (1) shows statistical analysis of serum levels of glucose (mg/dl), insulin (µU/ml), FSH (µU/ml), LH (µU/ml) and testosterone (ng/ml) in all studied groups. In Group Ib (normal obestatin treated group) there were no significant changes (p>0.05) in serum levels of glucose (68.87±2.90), insulin (9.04±0.50), FSH (0.48±0.04), LH (0.42±0.04) and testosterone (4.80±0.31) when compared with that of Group IA (normal vehicle treated group) (70.75±3.53, 8.85±0.716, 0.52±0.04, 0.40±0.02 and 4.85±0.28 respectively). Concerning effect of obestatin treatment in STZ-induced diabetic group (Group IIB), there were a significant increase in the mean values of serum levels of insulin (6.24±2.54, p<0.001), FSH (0.39±0.03, p<0.001), LH (0.37±0.09, p<0.001) and testosterone (3.95±0.28, p<0.001) accompanied by significant reduction in serum glucose levels (192.37±10.97, p<0.001) when compared with that of STZ-induced diabetic group (Group IIA) (2.17±0.18, 0.24±0.04, 0.20±0.02, 2.87±0.29 and 473.75±40.27 respectively). In addition, STZ-induced diabetic group (Group IIA) showed significant decrease in the mean values of serum levels of insulin, FSH, LH and testosterone associated with significant increase in serum glucose levels when compared with that of normal rats (Group IA) (p<0.001).

Table (2) represents the statistical analysis of testis wt (gm), epidydimal sperm count (millions/ml), epidydimal sperm motility (%) and percentage of deformed sperm in all studied groups. In Group IB (normal obestatin treated group) there was a significant increase in the mean values of testis weight (1.27±0.137 p<0.01) and epidydimal sperm count (51.75±4.06, p<0.01), but there were non-significant change in the mean values of percentage of deformed sperm (1.09±0.09, p>0.05) and epidydimal sperm motility (72.87±2.99, p>0.05) when compared with that of Group IA (1.09±0.16, 47.25±3.45, 1.55±0.15 and 69.62±3.92 respectively).

Regarding effect of obestatin treatment in STZ-induced diabetic Group (Group IIB), there were a significant increase in the mean values of testis weight (1.20±0.05, p<0.001), epidydimal sperm count (42.27±1.86, p<0.001) and epidydimal sperm motility (61.87±3.39, p<0.001), together with a significant reduction in the mean values of the percentage of deformed sperm (3.56±0.5 p<0.001) when compared with that of Group IIA (STZ-induced diabetes) (0.92±0.07, 24.24±1.73, 32.11±3.94 and 11.04±2.46 respectively). In addition,
STZ-induced diabetic group (Group IIA) showed a significant decrease in the mean values of testis weight ($p<0.01$, $p<0.001$), epididymal sperm count ($p<0.001$), epididymal sperm motility ($p<0.001$) and percentage of deformed sperm ($p<0.001$) when compared with both Group IA and IB.

Table (3) shows the statistical analysis of testicular MDA level (nmol/gm tissue), SOD activity (U/gm tissue), GPX activity (U/gm tissue) and CAT activity (U/gm tissue) in all studied groups. In Group IB there were a significant ($p<0.001$) increase in the mean values of testicular SOD activity (121.75 ± 5.45), GPX activity (34.45 ± 3.85) and CAT activity (44.00 ± 4.03) together with a significant reduction in testicular levels of MDA (124.85 ± 5.62) accompanied by significant decrease in testicular SOD activity, GPX activity and CAT activity to a greater extent in comparison to that of Group IA ($p<0.001$) and Group IIA ($p<0.001$). In Group IB, chronic obestatin treated, we found elongated seminiferous tubules lined by many layers of spermatogenic cells and excess number of mature sperms. In Group IIA (STZ-induced diabetic group) there were atrophy in the walls of seminiferous tubules with variable size and shape and reduction of sperm, Lineage cells. Moreover, walls of tubules were vacuolated and show destruction of sertoli cells with increased thickness of the basement membrane and edematous interstitial tissue. Histopathological examination:

Microscopic picture in rat testis (LM, H & E, focus 400) revealed: In Group IA (control), normal testis formed of uniform seminiferous tubules surrounded by thin basement membrane and lined by normal layers of spermatogenic cells up to mature sperm formation. In Group IB, chronic obestatin treated, there were elongated seminiferous tubules lined by many layers of spermatogenic cells and excess number of mature sperms. Group IIA (STZ-induced diabetic group) there were atrophy in the walls of seminiferous tubules with variable size and shape and reduction of sperm, Lineage cells. Moreover, walls of tubules were vacuolated and show destruction of sertoli cells with increased thickness of the basement membrane and edematous interstitial tissue. Regarding chronic obestatin treated STZ-induced diabetic Group (Group IIB) there were nearly seminiferous tubules equal in size and shape, in different stages of spermatogenesis with matured spermatozoids in the lumen, and normal sertoli cells. There is no edema in interstitial tissue, partially thick basement membrane, interstitial cells of leydig were normal.

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**Table (1): Statistical analysis of serum levels of glucose (mg/dl), insulin (µU/ml), FSH (µU/ml), LH (µU/ml) and testosterone (ng/ml) in all studied groups (A=Versus Group IA, B=Versus group IB, C=Versus group IIA, $p<0.05$ is considered significant).**

<table>
<thead>
<tr>
<th></th>
<th>Normal rats</th>
<th>STZ-induced diabetes</th>
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<tbody>
<tr>
<td></td>
<td>Group IA</td>
<td>Group IB</td>
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<tr>
<td></td>
<td>Group IIA</td>
<td>Group IIB</td>
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<tr>
<td><strong>Glucose (mg/dL):</strong></td>
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<tr>
<td>Mean ± SD</td>
<td>70.75±3.53</td>
<td>68.87±2.90</td>
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<tr>
<td>$p$-value</td>
<td>0.85</td>
<td>0.000$^{a,b,c}$</td>
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<tr>
<td><strong>Insulin (µU/ml):</strong></td>
<td></td>
<td></td>
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<tr>
<td>Mean ± SD</td>
<td>8.85±0.716</td>
<td>9.04±0.50</td>
</tr>
<tr>
<td>$p$-value</td>
<td>0.782$^a$</td>
<td>0.000$^{a,b,c}$</td>
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<tr>
<td><strong>FSH (µU/ml):</strong></td>
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<tr>
<td>Mean ± SD</td>
<td>0.52±0.04</td>
<td>0.48±0.04</td>
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<tr>
<td>$p$-value</td>
<td>0.11$^a$</td>
<td>0.000$^{a,b,c}$</td>
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<tr>
<td><strong>LH (µU/ml):</strong></td>
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<tr>
<td>Mean ± SD</td>
<td>0.40±0.02</td>
<td>0.42±0.04</td>
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<tr>
<td>$p$-value</td>
<td>0.402$^a$</td>
<td>0.000$^{a,b,c}$</td>
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<tr>
<td><strong>Testosterone (ng/ml):</strong></td>
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<tr>
<td>Mean ± SD</td>
<td>4.85±0.28</td>
<td>4.80±0.31</td>
</tr>
<tr>
<td>$p$-value</td>
<td>0.726$^a$</td>
<td>0.000$^{a,b,c}$</td>
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</table>
Table (2): Statistical analysis of testis weight (gm), epidydimal sperm count (millions/ml), epidydimal sperm motility (%) and percentage (%) of deformed sperm in all studied groups (A=Versus Group IA, B=Versus Group IB, C=Versus Group IIA, p<0.05 is considered significant).

<table>
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<tr>
<td></td>
<td>Group IA</td>
<td>Group IB</td>
</tr>
<tr>
<td>Testis wt (gm):</td>
<td></td>
<td></td>
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<tr>
<td>Mean ± SD</td>
<td>1.09±0.16</td>
<td>1.27±0.137</td>
</tr>
<tr>
<td>p-value</td>
<td>0.004</td>
<td>0.005</td>
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<tr>
<td>Epidydimal sperm count (millions/ml):</td>
<td></td>
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<tr>
<td>Mean ± SD</td>
<td>47.25±3.45</td>
<td>51.75±4.06</td>
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<tr>
<td>p-value</td>
<td>0.005</td>
<td>0.000a</td>
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<tr>
<td>Epidydimal sperm motility (%):</td>
<td></td>
<td></td>
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<tr>
<td>Mean ± SD</td>
<td>69.62±3.92</td>
<td>72.87±2.99</td>
</tr>
<tr>
<td>p-value</td>
<td>0.08</td>
<td>0.000a</td>
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<tr>
<td>Deformed sperm (%):</td>
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<tr>
<td>Mean ± SD</td>
<td>1.55±0.15</td>
<td>1.09±0.09</td>
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<tr>
<td>p-value</td>
<td>0.47</td>
<td>0.000a</td>
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Table (3): Statistical analysis of testicular MDA level (nmol/gm tissue), SOD activity (U/gm tissue), GPX activity (U/gm tissue) and CAT activity (U/gm tissue) in all studied groups (A=Versus Group IA, B=Versus Group IB, C=Versus Group IIA, p<0.05 is considered significant).

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<tbody>
<tr>
<td></td>
<td>Group IA</td>
<td>Group IB</td>
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<tr>
<td>Testicular MDA level (nmol/gm tissue):</td>
<td></td>
<td></td>
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<tr>
<td>Mean ± SD</td>
<td>109.01±3.82</td>
<td>71.43±7.24</td>
</tr>
<tr>
<td>p-value</td>
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<td>0.000a</td>
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<tr>
<td>Testicular SOD activity (U/gm tissue):</td>
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<tr>
<td>Mean ± SD</td>
<td>72.74±3.11</td>
<td>121.75±5.45</td>
</tr>
<tr>
<td>p-value</td>
<td>0.000a</td>
<td>0.000a</td>
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<tr>
<td>Testicular GPX activity (U/gm tissue):</td>
<td></td>
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<tr>
<td>Mean ± SD</td>
<td>24.35±2.51</td>
<td>34.45±3.85</td>
</tr>
<tr>
<td>p-value</td>
<td>0.000a</td>
<td>0.000a,b</td>
</tr>
<tr>
<td>Testicular CAT activity (U/gm tissue):</td>
<td></td>
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</tr>
<tr>
<td>Mean ± SD</td>
<td>18.74±1.77</td>
<td>44.00±4.03</td>
</tr>
<tr>
<td>p-value</td>
<td>0.000a</td>
<td>0.000a,b</td>
</tr>
</tbody>
</table>

Photo (1): Light photomicroscopic picture of rat testicular tissue isolated from normal rat (Group 1A) showing seminiferous tubules with compact epithelial cells which were arranged normally, normal basement membrane thickness (PM), presence of germ cells in different stages of spermatogenesis (S) and matured spermatozoids (MS) in the Lumen (H & E focus 400).

Photo (2): Light photomicroscopic picture of rat testicular tissue isolated from normal rat treated with obestatin (Group 1B) showing seminiferous tubules with an increase in the spermatogenic activity, apparent sperm lineage and matured spermatozoids in the lumen, and normal Sertoli Cells (SC). Thickness of the basement membrane (PM) and interstitial cells of leydig (LC) were normal (H & E focus 400).
Effect of Obestatin on Testicular Functions of Normal & STZ Induced Type 1 Diabetic Rats

Photo (3): Light photomicroscopic picture of rat testicular tissue isolated from STZ-induced diabetic rat (Group 1IA) showing seminiferous tubules with atrophy in their walls and reduction of sperm Lineage cells. Wall of tubules were Vacuolated (V) and shows also destruction of Sertoli Cells (SC). Basement Membrane (PM) thickness increased and became irregular. In addition to, oedema of the interstitial tissue (H & E focus 400).

Photo (4): Light photomicroscopic picture of rat testicular tissue isolated from STZ-induced diabetic rat chronically treated with obestatin (Group 1IB) showing seminiferous tubules in different stages of Spermatogenesis (S) with matured spermatozoids in the lumen, and normal Sertoli Cells (SC), partially thick basement membrane (PM) and normal interstitial cells of leydig (LC) (H & E focus 400).

Discussion

In a trial to estimate the impact of diabetes on testicular function, the present research used STZ-induced diabetes as a model of DM 1, it was found that STZ-induced diabetes (Group IIA), which had significant hyperglycemia and low insulin level compared to control rats (Group IA), resulted in a significant reduction in serum levels of FSH, LH, testosterone, in addition to the significant reduction of testes weight, epidydimal sperm count and epidydimal sperm motility with significant increase in the percentage of deformed sperm. Taken together (serum and epidydimal sperm finding) indicates hypo-fertility complication of uncontrolled DM 1.

This result supported by Ballester et al., [38], and Aquila et al., [39] who reported that in DM 1, leydig cell function and testosterone production decreased because of the absence of the stimulatory effect of insulin on these cells and an insulin-dependent decrease in FSH and LH levels. Moreover, as a consequence of reduced insulin action, Leydig cell function may be compromised and testicular steroidogenesis impaired, leading to decreased circulating testosterone concentrations [40].

Moreover, Bhattacharya et al., [41] Omu et al., [7] and Basmatzou and Hatziveis [42] reported significant presence of round and elongated spermatid in semen of men with insulin dependent diabetes and they explain that to be a result of dysregulation of the process of spermiation in which mature spermatozoa are normally released into the luminal compartment of the seminiferous tubule.

In addition to serum and epidydimal parameters, while testicular SOD, CAT and GPX enzymatic activity were significantly decreased, testicular MDA levels were significantly increased in STZ-induced diabetic group (Group IIA) indicates a state of Oxidative Stress (OS) behind testicular malfunctions. Diabetes has marked effects on normal cellular processes; as it potentiated disturbance of mitochondrial free radical production and imbalance between ROS generation and antioxidant defenses [43]. This OS constitutes major factors in the pathogenesis of most of the long-term diabetes dysfunction [44]. The increased damage in sperm DNA results from diabetic males whose sperm that is getting developed is exposed to huge levels of glucose and as a consequence to oxidative insults [45-47].

Our findings are supported also by the report of Annunziata et al., [48] who state that increased lipid peroxidation and the imbalanced carbohydrate metabolism in diabetes mellitus could cause impairment of the steroidogenic function of the testis in addition to the cellular and tissue damage. This may explain the possible etiologies for increasing cases of infertility among diabetic males [10,11,42], as diabetes mellitus particularly with poor glycemic control is associated with impaired sperm quality, involving oxidative stress in the pathogenesis. Antioxidant therapy has been shown to significantly
improve the sperm quality \[7,42,49\]. At the level of the testes, oxidative stress is capable of disrupting the steroidogenic capacity of Leydig cells \[50\] as well as the capacity of the germinal epithelium to differentiate normal spermatozoa \[51\], triggering germ cell apoptosis resulted in reduction in sperm count \[52\].

Beside increased oxidative stress, other factors as autoimmune disorder with the development of anti-spermatozoan antibodies and/or neuropathy which may alter seminal vesicle function and worse spermatozoa parameters, including motility in DM 1 patients \[13,53\].

Regarding chronic daily obestatin administration, in normal male albino rats; obestatin significantly elevated the testes weight, testicular SOD, CAT and GPx activity, together with significantly reduced testicular MDA levels and percentage of deformed sperm. In STZ-induced diabetic male albino rats, obestatin administration results in a significant increase in testes weight, serum levels of insulin, FSH, LH and testosterone, epidydemal sperm count, sperm motility, testicular SOD, CAT and GPx activity, together with significantly reduction in serum glucose levels, testicular MDA levels and percentage of deformed sperm.

The significant hypoglycemic effect of obestatin in this study is in line with the previous studies which concluded that obestatin prevented the development of diabetes mellitus symptoms and improved glucose metabolism in STZ-induced rat model of diabetes \[54,55\]. These data are supported by Alloatti et al., \[56\] who identified binding sites for the obestatin peptide in the pancreas, moreover, Granata et al., \[57,58\], Delhanty et al., \[59\], Favaro et al., \[60\] and Baragli et al., \[61\] concluded that obestatin has a role in regulating the cell cycle, exerting proliferative effects on β-cells and promoted cell survival in human isolated pancreatic islet cells and in the HIT-T15 and INS-IE pancreatic β-cell lines. Furthermore, obestatin reduced glucose levels as it enhanced insulin release in perfused rodent islets \[58,62,63\] β-cells were more responsive to obestatin when glucose levels were high \[62,64\].

In addition, obestatin prevents lipolysis and acts similarly to insulin, reduced insulin resistance and reduced inflammation in metabolic tissue \[58\]. However, obestatin was reported to have no effect on glucose or insulin levels under basal or fasting conditions in rats and mice \[65,66\].

The improvement in testicular function in the present work is matching with the ability of obestatin to enhance the testosterone production from the testes, oxidative stress is capable of disrupting the steroidogenic capacity of Leydig cells either directly through binding of obestatin to receptor (GPR 39) which is present in testes \[67\] or by enhancing the responsiveness of the leydig cells towards pituitary LH \[24\]. Moreover, the associated significant improvement in insulin levels proved in this research also act as indirect preservation of testicular functions, as insulin hormone is essential to stimulate Sertoli cells to synthesize and secrete androgen-binding protein, which is important to bind testosterone that is required for terminal differentiation of spermatids \[68\]. In addition, the associated increase in FSH levels would also increase the production of the stem cell factor from Sertoli cell. This stem cell factor has been involved in Leydig cell development and survival and is acting as a survival factor for the different cell types in the seminiferous epithelium such as spermatogonia in adult rats \[69\].

The obestatin treated rats (Group Ib and IIB) showed a significant decrease in serum MDA levels with significant increase in SOD, CAT, and PGx activities compared to that of untreated groups (Group IA and IIA). The reason might be due to the ability of obestatin to restore oxidative balance and decrease OS. This concept of obestatin to be able to exert protective effect against oxidative stress either by itself was concluded by Aragno et al., \[70\], or the cause might be attributed to the antioxidant properties of adiponectin \[71\] as obestatin induced phosphorylation of Adenosine Monophosphate Kinase (AMPK) which acts as substrate of phosphoinositide 3-kinase/Akt, extracellular signal-related kinase (PI3K/Akt, ERK) and protein kinase C (PKC), this will reduce inflammatory markers such as TNF-α and IL-6 \[70,72\] and lead to induction of adiponectin which correlated positively with obestatin and improved insulin sensitivity and increasing energy expenditure and fatty acid oxidation \[73,74\].

**In conclusion:**

Chronic obestatin administration has beneficial effects regarding glucose metabolism as it has hypoglycemic and insulin secretion stimulatory activity in STZ induced diabetic rats. Moreover, obestatin owns antioxidant properties and can guard against diabetes-induced testicular dysfunction and successes in improving the hypo-fertility state of uncontrolled diabetes. Taken together, it could be suggested that obestatin has potential therapeutic perspectives in diabetic metabolic and gonadal dysfunctions.

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تأثير الأوبيستاتين على وظائف الخصية في الجرذان السليمة والمصابة

داء السكري هو خلل أيضي يفضي إلى العديد من المضاعفات الخطيرة تتطلب بحالة الأكسدة المزدوجة، هرمون الأوبيستاتين من الببتيدات التي تنتجها الأنسجة المختلفة، وقد ثبت وجوده وفعلاً في الخصية كما أن الآحاد الحديث قد نسبت خصائص مضادة للأكسدة لهذا البيتل.

الهدف من البحث: وقد تم تصميم هذا البحث لتحقق اثر مرض السكري غير معالج ووظائف الخصية في مجموعات الجرذان المصابة بالنوع الأول من داء السكري (المعروف بالأنسولين) المحدث بالاسترويدزوسين. كما يهدف البحث إلى دراسة دور الأوبيستاتين على وظائف الخصية سواء في الجرذان السليمة أو المصابة بداء السكري المحدث بالاسترويدزوسين.

مواد وطرق البحث: أجريت التجربة على 20 من ذكور الجرذان البضاء (314±93جم) التي قسمت عشوائياً على قسمين على ما يلي:

- مجموعة (أ): حصلت من خلال البروتون بجرعة 100 ميكروجرام من محلل تركيز 500 / يوماً لمدة 20 يوماً مرتين، ومجموعة (ب): تلقى حصل من خلال البروتون بجرعة 100 ميكروجرام/100 جرام من وزن الجسم لمدة 20 يوماً مرتين.

و44 ساعة بعد إخضاع مجموعة البايتين تم زرع عينات الدم من جزء الأوردة خلف العين وفصل محلل الدم منها، وقد تم قياس مستويات الجلوكوز والأنسولين وهرمون ترسيب جزيئات المبادئ وهرمون الوتة وهرمون الاسترويدزوسين في محلل دم الجرذان. تم قياس نسبة الاملأ الدانتي القياس 40% إنتاج الملوثات وقياس نسبة النشاط للمتنازلات من إنزيمات الورابوكسيدين، وبذارتين، والجمليات، والكالكالاز، والكلوروكسيدين، وتم مراقبة الخصية الدماغية بالبيوكسيدين الضوئي.

وقد أسفر البحث عن النتائج التالية:

أن مرض السكري المحدث بعلام الاسترويدزوسين قد أظهر إفرازات داخلية في وزن الخصية، ومستويات الأنسولين وهرمون ترسيب جزيئات المبادئ وهرمون الاسترويدزوسين في محلل دم الجرذان، كما ثبت أن الفرز الإحصائي يتقلص في قد خصائص الحيوانات المنوية بالحمى. ونتيجة للعملية مع زيادة نسبة التكسير، بما دلالة إنشاء جديد إحدى إنزيمات الورابوكسيدين، ديبويزوتان والكلكالاز وزالكلوروكسيدين، جنبًا إلى جنب مع تغييرات في حالة الأنسجة الخصية بالخصية المحرجة.

وقد، بعد أن التأكد من أن التأثير هو في الأنسولين أدى إلى تحسين كبير لدارة إحصائية في جميع الببتيدات المذكورة أعلاه في المجموعات المصابة بالسباب. ونتيجة، بما في المجموعة السليمة المحترقة بالأوبستاتين قد أجبرت ثغرات ذات دلالة إحصائية في زيادة مضادات الأكسدة بالنسبة لمجموعة الفضلات

عن زيادة في وزن الخصية مع زيادة في حيوانات المنوية.

الخلاصة: نظراً لإمكانيات الأوبيستاتين لخصائص مضادة للأكسدة وقطرة في تقليل مستوى الجلوكوز مع رفع ثغرات الأنسولين فإنها دواعي قوية ضد ضعف ووظائف الخصية التي يسببها مرض السكري. وعليه فإن استخدام الأوبيستاتين يمكن أن يحسن الأيض ويحمي من أضرار مرض السكري المفعول إلى نفس الخصية.