Atorvastatin and Zinc Combination Mitigates High Fat Diet-Induced Biochemical and Histopathological Changes in the Testis of Male Albino Rat

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ABSTRACT

Hyperlipidemia is a crucial factor in the pathogenesis of infertility. Atorvastatin (AT) is the first line of treatment for dyslipidemias having anti-inflammatory, antioxidant and testicular protective effects. However, decreased testosterone and sperm quality are reported. Zinc is an essential trace element for the oxidant defense system, DNA replication, protein synthesis, spermatogenesis, and steroidogenesis. These are contradictory data regarding AT impacts on the testis. This study aims to assess the possible protective effects of AT and Zinc solely or combined in HFD-induced testicular injury.

Forty male albino rats were allocated into five groups: control group, group (H) received HFD, group (HA); received HFD and AT, group (HZ); received HFD and Zinc, group (HAZ); received HFD, AT and Zinc for 8 weeks. Administration of AT and Zinc together or separately significantly improves body and testicular weights together with the significant recovery in biochemical and hormonal assessment. Group (H) showed disorganized atrophic seminiferous tubules with losses, thickness reduction and focal separation of spermatogenic epithelium. Scarce spermatozoa, hyalinized material and vacuolations appeared in the lumina. Groups (HZ) and (HAZ) showed more improvement in the histological architecture. Moreover, HAZ rats demonstrated the normal distribution of collagen fibers and strong positive PCNA immunoreactivity and that was confirmed morphometrically. HFD exhibited deleterious functional and structural alterations in the testes of male albino rats. AT alone was proved to have controversial effects on the testicular histological structure. These alterations could be attributed mainly to HFD ± AT. Accordingly, I highly recommended the concomitant administration of Zinc with AT to alleviate these deleterious changes.

INTRODUCTION

According to the World Health Organization (WHO) more than half a billion adult individuals are suffering from obesity and approximately three million die because of its complications per year (World Health Organization, 2018). Improvement of the life standard due to the fast development of the social economy and productivity has a great impact on the dietary pattern in the form of regular ingestion of high-sugar, high-fat and high-protein foodstuffs. Subsequently, disturbance in lipid metabolism, reduction in the antioxidant capacity, increased end products of lipid oxidation and finally atherosclerosis (Ye et al., 2014).
Obesity, caused by excessive lipid ingestion regardless of its type, is usually linked to increased incidence of several disorders such as insulin resistance, type 2 diabetes mellitus, dyslipidemia, hypertension, cancer and reproductive disturbance (Fernandez et al., 2011). In male reproductive parameters, numerous researchers have demonstrated that the body mass index has a direct relationship to hyperleptinemia. Hypogonadism in obese males could be attributed to an increase in leptin that in its turn lowers testosterone levels (Michalakis et al., 2013). Moreover, the high-fat diet (HFD) impinges on the structure of the cellular plasma membranes and alters the receptors’ availability. In the testes, alterations in the gonadotropin (LH and FSH) receptors’ availability lead to fluctuations in testosterone production and affection of the spermatogenesis (Mah and Wittert, 2010).

Hyperlipidemia, due to lipid metabolism disorder, can be in the form of increased total cholesterol, triglycerides and/or low-density lipoprotein cholesterol levels, and/or decreased levels of high-density lipoprotein protein cholesterol (Haibin and Zhenling, 2010). Hyperlipidemia is a crucial factor in the pathogenesis of atherosclerosis, coronary heart disease, myocardial infarction, stroke, diabetes complications, fatty liver and infertility (Gao et al., 2017).

Therefore, the development of drugs for dyslipidemias becomes a must (Mostafa et al., 2015). Statins (atorvastatin, simvastatin, lovastatin, pravastatin, rosuvastatin, and Fluvastatin) are one of the most commonest approved drugs used in treating dyslipidemias (Sonar, 2013). It acts by inhibiting the activity of the 3-hydroxy-3-methyl glutaryl coenzyme A reductase (HMG-CoA reductase), which is a main enzyme in the cholesterol biosynthetic (Mostafa et al., 2015). In addition to the previous action, various clinical and experimental investigations demonstrated anti-inflammatory, neuroprotective, nephroprotective (Song, Li and Cai, 2004), cardioprotective (Shida et al., 2014), immunomodulatory, and antioxidant effects, (Yang et al., 2017) as well as the protective effect in hindering the testicular damage induced by obesity (Cui et al., 2017). Statins exert their antioxidant action by cleaning the superoxide and hydroxyl free radicals and hence decreasing the cellular damage (Miwa et al., 2005). Also, prevent hydrogen peroxide induced-apoptosis due to lipid peroxidation in the endothelial cells (Xu et al., 2008).

Atorvastatin (AT) is the commonest prescribed statin and is considered the proper first line of treatment of dyslipidemias in susceptible patients who are at risk of coronary heart disease (Solomon and Freeman, 2008). However, serious side effects of statins are reported such as muscle injury, dose-dependent elevations in liver enzymes, decreased free testosterone levels and reduced sperm quality (Pons-Rejraji et al., 2014). Moreover, it enhances oxidative stress by increasing the synthesis of reactive oxygen species (ROS) (Pal et al., 2015).

Zinc (Zn) is an essential trace element for a wide range of biological activities, including the oxidant defense system (Al-Fartusie and Mohssan, 2017), also it is required for the activities of more than 300 enzymes and 2000 transcription factors (Maremanda and Jena, 2017). Moreover, It plays an imperative role in DNA replication, transcription and protein synthesis thus promoting cellular division and differentiation (Askary et al., 2011). Additionally, it hinders cellular damage via the activation of the antioxidant pathways (Payahoo et al., 2013). Therefore, dietary Zn deficiency promotes lipid peroxidation and cellular damage in numerous tissues including the testis (Zhao et al., 2011). These impairments could be reversed by Zn supplementation (Rahman et al., 2019). The beneficial effects of Zn in male
Atorvastatin and Zinc synergistically ameliorate High Fat Diet-Induced Testicular injury

reproduction in the maintenance of spermatogenesis and steroidogenesis (Fallah, Mohammad-Hasani and Colagar, 2018) are well recognised. In the testis, Zn is crucial for the process of sperm formation, maturation and stabilization of condensed sperm chromatin (Cheah and Yang, 2011).

In Animal studies, Zn deficiency increases the susceptibility of the vascular endothelial cells to the harmful impacts of oxidative stress as it negatively affects its histological structure, fatty acid and carbohydrate metabolism (Beattie et al., 2008). Furthermore, in human studies, a strong negative correlation was detected between Zn supplementation and the incidence of hyper-triglyceridemia, hypertension, diabetes and cardiac diseases. Moreover, Zn supplementation may prevent atherogenesis by lowering total cholesterol, triglycerides, and low-density lipoprotein and increasing the level of high-density lipoprotein (Hashemipour et al., 2009).

The Aim of The Study:

There are contradictory data regarding the beneficial or hazardous impacts of AT in HFD-induced testicular injury in rats. Additionally, Zn is well-reported to have an imperative role in spermatogenesis and steroidogenesis. Hence, the present study aims to assess the possible protective and therapeutic effects of AT and Zn solely or combined in HFD-induced testicular injury and to clarify the underlying mechanisms. Therefore, laboratory, histological and immunohistochemical analyses were carried out to investigate these effects.

MATERIALS AND METHODS

Animals:

Forty adults male Sprague-Dawley albino rats aged 12 weeks weighing 200–250 gm were used in the experiment. They were obtained from the Animal Breeding Unit of Taibah University, Kingdom of Saudi Arabia. The animals were housed in well-ventilated hygienic cages under the standard conditions at (12:12 h light/dark cycle, room temperature of 24 - 28° C and humidity at 50–60%) with free access to water and a standard balanced diet. All procedures were accepted by “The Research Ethics Committee”, College of Medicine, Taibah University, Saudi Arabia. This was strictly conducted in compliance with the guidelines as recommended by the Science Council of Japan or the National Research Council’s criteria (NIH No. 86-23).

Experimental Design:

The animals were allocated randomly into five groups eight animals in each; the control (group C) allowed ad libitum access to tap water and chow for 8 consecutive weeks. HFD (group H) received a diet containing sheep tail fat in a dose of 500mg /kg daily via gastric gavage for 8 weeks (Cimen et al., 2017). The HFD and AT (group HA); received HFD as previously mentioned and AT (Lipitor tablet, SIGMA; Pharmaceutical Industries-Egypt). The AT tablet (80 mg) was dissolved in 40 ml of distilled water and each rat was given 0.72 ml (containing 1.44 mg of atorvastatin) orally via a gastric tube once daily for 8 weeks (Mostafa et al., 2015). The HFD and Zn (group HZ); received HFD as mentioned above and Zn (zinc supplement capsules, Thompson, nutritional, USA) in a dose of 30 mg/kg orally for 8 weeks via gastric tube (Gebchag et al., 2019). The HFD, AT and Zn (group HAZ); received HFD, AT and Zn as described above for 8 weeks.

Specimen Collection:

At the end of the experimental period, the rats were weighed, and fasted overnight and blood samples were obtained from the retro-orbital venous plexus, collected in dry sterile tubes and allowed to clot for 60 min. Sera were obtained by centrifugation at 3000 x g for 15 minutes and stored at -70 °C until the time of analysis. All rats were sacrificed under light ether anesthesia. Testes were quickly excised and weighed. The tissues from the right testes were processed for biochemical analyses. On the other hand, the left testes were handled for
histological and immunohistochemical studies (El-Mehi and Faried, 2019).

**Assessment Methods:**

**Total Body and Testicular Weight Assessment:**

The total body weight and testicular weight were reported at the end of the experiment.

**Biochemical Assessment:**

**Lipid Profile Assessment:**

Lipid profiles including serum cholesterol (CH), triglycerides (TG), low-density lipoprotein (LDL) and high-density lipoprotein (HDL) were assessed using the commercially available kits (BioDiagnostic Company, Dokki, and Giza, Egypt, catalogue No. CH 12 20, TR 20 30, CH 12 31, CH 12 30) and manipulated according to the manufacturer’s instructions.

**Hormonal Indices Assessment:**

Serum levels of luteinizing hormone (LH) & testosterone were measured by an Enzyme-Linked Immunosorbent Assay (ELISA) by using the available commercial kits (DRG ELISA Kit, Germany catalogue No. EIA-1289 and EIA-1559 respectively).

**Lipid Peroxidation and Tissue Antioxidant Status:**

The right testes of rats were immediately frozen and stored in liquid nitrogen at -70 °C. Frozen tissue was homogenized in ice-cold phosphate buffer (KCl 140 mmol/l, phosphate 20 mmol/l, pH 7.4) and centrifuged at 10000 × g for 15 minutes. The supernatant was used for the measurement of tissue activities of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPX) and malondialdehyde (MDA) (El-Mehi and Faried, 2019).

**Histological Examination:**

**Light Microscopic Study:**

The testicular specimens were immediately fixed by immersion in Bouin’s solution for 24 h, then dehydrated in ascending grades of ethyl alcohol, cleared in benzene, and then embedded in paraffin wax. The processed blocks were cut into sections 5 microns thick by the rotatory microtome and stained with hematoxylin and eosin (H&E), Masson’s trichrome (MT) and immunohistochemical staining for anti-proliferating cell nuclear antigen (PCNA).

**Immunohistochemical Staining:**

The deparaffinized and rehydrated 5-μm sections were rinsed with phosphate buffer solution (PBS), blocked in 3% H₂O₂ as an inhibitor of endogenous peroxidase activity. After rinsing in PBS, the microwave antigen retrieval procedure was performed. The sections were then incubated with the primary antibody: PCNA (1:100, mouse monoclonal, Abcam), at room temperature for an hour. Sections were rinsed with PBS, followed by 20 min of incubation at room temperature with a secondary biotinylated antibody. After rinsing the sections in PBS, enzyme conjugate “Streptavidin-Horseradish peroxidase” solution was applied to the sections for 10 min. Secondary antibody binding was visualized using 3,3-diaminobenzoic acid (DAB). Finally, sections were PBS rinsed and counterstaining of slides was done using two drops of hematoxylin (El-Mehi and Faried, 2019).

**Digital Morphometric Assessment:**

Slides were photographed using Olympus® digital camera installed on an Olympus® microscope with a 1/2 X photo adaptor, using 20X objective. The result images were analyzed on Intel® Core I7® based computer using Video Test Morphology® software (Russia) with a specific built-in routine for the area, % area, measurement, object counting and Contact Angle. Thirty cross-sections for each animal from the different groups were assessed for the diameters (in different angles) and perimeters of the seminiferous tubules (El-Mehi and Faried, 2019). The data were then expressed as mean and standard deviation (Fig. 1 A, B).

The area percentage of MT staining and PCNA immunoreaction were calculated by examining ten non-overlapping fields (20x) for every specimen, randomly taken from the
Atorvastatin and Zinc synergistically ameliorate High Fat Diet-induced Testicular injury. The rats of group (H) showed a significant increase (p<0.001) in total body weight when compared to group (C). Administration of AT and Zn together or separately significantly decreased the total body weight when compared to the group (H) (Table 1).

The testicular weights were significantly decreased (p<0.001) in group (H) when compared to group (C). In the other groups (HA, HZ and HAZ) the testicular weight exhibited a significant increase (p<0.001) as compared to the group (H) (Table 1).

**RESULTS**

**Body Weight and Testicular Weight:**

Table 1: Comparison between various groups regarding total body and testicular weights (gm).

<table>
<thead>
<tr>
<th></th>
<th>Group C</th>
<th>Group H</th>
<th>Group HA</th>
<th>Group HZ</th>
<th>Group HAZ</th>
<th>P value</th>
</tr>
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<tbody>
<tr>
<td>Total body weight</td>
<td><strong>274.80±10.52</strong></td>
<td><strong>315±8.72</strong></td>
<td><strong>289.60±6.23</strong></td>
<td><strong>304±5.24</strong></td>
<td><strong>286.8±8.38</strong></td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Testis weight</td>
<td><strong>1.99±0.12</strong></td>
<td><strong>1.31±0.13</strong></td>
<td><strong>1.85±0.07</strong></td>
<td><strong>1.72±0.11</strong></td>
<td><strong>1.97±0.10</strong></td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

Statistical Analysis:

Statistical analysis of the data obtained from the various studied groups was expressed as mean values and standard deviations, and statistical significance was determined by one-way analysis of variance (ANOVA) followed by *Tukey’s post hoc* test for multiple comparisons. Differences were considered statistically significant at (P < 0.001).

**Lipid Profile Assessment:**

As compared with the control group, analysis of the sera of rats in group (H) revealed that the levels of CH, TG, and LDL significantly increased (p < 0.001). Moreover, the level of HDL significantly decreased (p < 0.001), indicating that the hyperlipidemic state was reached and the target model was successful (Nawaz et al., 2017). Compared to group (H), HAZ group demonstrated a significant decrease in the serum levels of CH, TG, and LDL (P < 0.001) and a significant increase in HDL levels (P < 0.001) (Table 2).

**Hormonal Indices Assessment:**

Serum levels of LH and testosterone significantly decreased (P < 0.001) in group (H) in comparison with group (C). Treating the rats with both AT and Zn resulted in a significant increase in the LH and testosterone levels (P < 0.001) compared to the group (H) (Table 2).

**Lipid Peroxidation and Tissue Antioxidant Status:**

The concentrations of SOD, CAT and GPX were significantly decreased (p<0.001) in the homogenized testicular tissues of group (H) when compared to group (C). However, a significant increase (p<0.001) in the MDA concentrations compared to the control rats was detected. Treating the rats with AT and Zn together resulted in a significant increase in SOD, CAT and GPX (p<0.001), and a significant decrease in the MDA levels (p<0.001) in comparison with the group (H) (Table 3).
Table 3: Oxidative stress markers assay.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Group C</th>
<th>Group H</th>
<th>Group HA</th>
<th>Group HZ</th>
<th>Group HAZ</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAT (Unit/mg/protein)</td>
<td>17.48±1.56</td>
<td>6.97±0.60</td>
<td>12.04±1.95</td>
<td>9.54±0.24</td>
<td>14.28±0.9</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>SOD (Unit/mg/protein)</td>
<td>72.85±2.95</td>
<td>33.07±2.26</td>
<td>56.67±7.41</td>
<td>43.31±3.93</td>
<td>60.87±1.77</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>GPX (umol/mg protein)</td>
<td>134.37±7.08</td>
<td>49.94±4.47</td>
<td>98.69±8.65</td>
<td>76.69±4.17</td>
<td>123.47±4.35</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>MDA (nmol/mg protein)</td>
<td>1.33±0.07</td>
<td>2.45±0.10</td>
<td>1.84±0.12</td>
<td>1.99±0.08</td>
<td>1.60±0.12</td>
<td>&lt;0.001*</td>
</tr>
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a: Significance vs group C, b: Significance vs group H, c: Significance vs group HZ, d: Significance vs group HAZ

Histological Assessment:
H&E-Stained Sections:

Light microscopic examination of H&E-stained sections of the testes of group (C) exhibited normal histological architecture. The testes were composed of densely packed well-organized seminiferous tubules separated from each other by a thin interstitium (Fig.2A). The seminiferous tubules were lined by stratified spermatogenic cells of several stages of differentiation. Each tubule had tiny basal lamina, multiple rows of spermatogonia, primary spermatocytes, spermatids and Sertoli cells. (Fig.2A and Fig.3A). The first rows resting on the basal lamina were the spermatogonia. Two types of spermatogonia were demarcated, oval type A with large oval lightly stained nuclei and rounded type B with rounded deeply stained nuclei (Fig.3A). Perpendicular to the basal lamina, the supporting Sertoli cells, appeared columnar in shape containing elongated lightly stained basophilic nuclei (Fig.3A). The largest cells were primary spermatocytes, rounded in shape and contained large rounded deeply stained basophilic nuclei (Fig.3A). Three to four rows of spermatids were observed having nuclei varying from rounded lightly stained to elongated deeply stained (Fig.2A and Fig.3A). Spermatozoa were noticed in the lumen of the tubules (Fig.2A and Fig.3A). Scanty non-congested blood vessels, loose connective tissue, and clusters of the interstitial cells of Leydig were demarcated in the interstitium intervening the tubules (Fig.2A and Fig.3A).

The testes of group (H) showed atrophic with irregular and interrupted basal lamina (Fig.2B). The spermatogenic epithelium showed several degenerative changes in the form of losses, reduction in their thickness, wide intercellular spaces and focal separation from the basal lamina. The germinial cells displayed vacuolated cytoplasm and their nuclei showed variable changes such as fading of its basophilia, deep staining, shrinkage and fragmentation (Fig.3B). Scarce spermatozoa, acidophilic hyalinized material and numerous vacuolations appeared in the lumina of the tubules. The interstitium appeared widened with multiple vacuolations, congestion of the capillaries and deposition of homogenous acidophilic material. Various interstitial cells of Leydig were degenerated and had shrunken deeply stained basophilic nuclei (Fig.2B, Fig.3B).

In group (HA), improvement in the histological architecture as compared to group (H) was observed. However, the thickening of the capsule and congestion of the blood vessels were still noticed (Fig.2C). Also, within the seminiferous tubules, vacuolation, thinning of the lining spermatogenic cells and nuclear degenerative changes were demonstrated (Fig.3C). The lumina of several tubules contained few spermatozoa (Fig.2C and Fig.3C). Moreover, minimal eosinophilic material in the interstitium was still present (Fig.2C).

Histological examination of the testes of the group (HZ) showed more improvement in the histological architecture as compared to group (H). Though, minimal congestion of the blood capillaries and widening of the interstitium were still observed.

The testes of group (H) showed atrophic with irregular and interrupted basal lamina (Fig.2B). The spermatogenic epithelium showed several degenerative changes in the form of losses, reduction in their thickness, wide intercellular spaces and focal separation from the basal lamina. The germinial cells displayed vacuolated cytoplasm and their nuclei showed variable changes such as fading of its basophilia, deep staining, shrinkage and fragmentation (Fig.3B). Scarce spermatozoa, acidophilic hyalinized material and numerous vacuolations appeared in the lumina of the tubules. The interstitium appeared widened with multiple vacuolations, congestion of the capillaries and deposition of homogenous acidophilic material. Various interstitial cells of Leydig were degenerated and had shrunken deeply stained basophilic nuclei (Fig.2B, Fig.3B).

In group (HA), improvement in the histological architecture as compared to group (H) was observed. However, the thickening of the capsule and congestion of the blood vessels were still noticed (Fig.2C). Also, within the seminiferous tubules, vacuolation, thinning of the lining spermatogenic cells and nuclear degenerative changes were demonstrated (Fig.3C). The lumina of several tubules contained few spermatozoa (Fig.2C and Fig.3C). Moreover, minimal eosinophilic material in the interstitium was still present (Fig.2C).

Histological examination of the testes of the group (HZ) showed more improvement in the histological architecture as compared to group (H). Though, minimal congestion of the blood capillaries and widening of the interstitium were still observed.
Moreover, the seminiferous tubules demonstrated areas of loss and few spermatozoa within its lumina. Also, very minimal interstitial eosinophilic materials were still present (Fig.2D and Fig.3D).

However, the rats of the group (HAZ) demonstrated noticeable improvement in the histological structures of the testes approaching that of the control group. Also, we noticed marked reversibility of the histopathological changes as compared to the group (H) (Fig.2E and Fig.3E).

**Masson Trichrome Sections:**

The testes of the control group were covered by a thin capsule composed of two layers; tunica albuginea and tunica vasculosa. Normal distribution of collagen fibers was demonstrated in the capsular element and wall of blood vessels (Fig.4A). In groups (H, HA and HZ), the capsule became thicker with marked deposition of collagen fibers and also exhibited dilated and congested blood vessels. (Fig 4B, C, D). However, in HAZ rats, more or less normal distribution of the collagenous fibers was observed in the capsule and around the wall of mildly congested blood vessels (Fig. 4E).

![Fig.1: Photomicrographs of section in the testis demonstrating the way of measuring the diameters (A) and the perimeters (B) of the seminiferous tubules (H&E 200x, scale bar=50 μm).](image-url)
Fig. 2: Photomicrograph of H&E-stained transverse section of the testis of the different groups. 

A: group (C) shows normal well organized, compact seminiferous tubules separated by narrow interstitium containing aggregation of Leydig cells (LC). The tubules are lined with normally arranged spermatogenic epithelial cells; spermatagonia (SG), primary spermatocytes (Sp) and spermatids (Sd). Sertoli cells (SC) are noticed among the spermatogenic cells resting on the basement membrane. Spermatozoa (SZ) are also well seen in the lumen of the tubules. 

B: group (H) shows disorganized and disrupted seminiferous tubules lined with degenerated spermatogenic epithelium. Loss of spermatogenic cells (thick arrow) and decreased its thickness are obviously demonstrated. Scarce or even no spermatozoa (double arrowhead), acidophilic hyalinized material and numerous vacuolations (V) appear in the lumen of the tubules. The tubules are separated by wide interstitium containing multiple vacuolations (arrow), dilated blood vessel (BV) and homogenous acidophilic material (*) and degenerated Leydig cells (tailed arrow). 

C: group (HA): shows slight improvement in the histological structure when compared to group (H) apart from capsular thickening and dilated congested blood vessel (arrow). The thickened capsule is formed of two layers; tunica albuginea (TA) and tunica vasculosa (TV). Some interstitial cell of Leydig appear degenerated (LC). Minimal acidophilic material (arrowhead) is seen within the interstitium. The spermatogenic cells show decrease thickness with areas of loss. Few sperms (star) and vacuolations are seen in the lumen of the tubules. 

D: group (HZ) showed more improvement in the histological architecture as compared to group (H). Mildly congested blood capillaries (arrowhead) and minimal interstitial acidophilic materials (*) are defined in the interstitium. Some Leydig cells have normal appearance (arrow). The tubules are lined with several layers of spermatogenic cells with areas of loss (wavy arrow) and focal separation from basal lamina (crossed arrow). Thickening of the capsule is also detected and composed of tunica albuginea (TA) and tunica vasculosa (TV). 

E: group (HAZ) demonstrates noticeable improvement in the histological structures of the testes approaching that of the control group. Regularly organized seminiferous tubules are observed. The spermatogenic cells are arranged in multiple rows with different stages of differentiation; spermatagonia (SG), spermatocytes (Sp), spermatid (Sd) and spermatozoa (SZ). Normal Sertoli (SC) and Leydig cells (LC) are also well shown (H&E 200x, scale bar=50 μm).
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**Fig. 3:** Representative micrographs of H&E-stained testicular sections of the various groups. 

A: **group (C)** shows normally arranged seminiferous tubules separated by narrow interstitium containing Leydig cells (LC). The tubules are lined with 7-8 rows of spermatogenic cells: spermatogonia (SG), primary spermatocytes (Sp) and spermatids (Sd). Sertoli cells (SC) are noticed resting on the basal lamina. Spermatozoa (SZ) are well seen in the lumen of the tubules. 

B: **group (H)** shows that the disrupted seminiferous tubules are lined with 2-3 rows of spermatogenic epithelium. The spermatogenic cells show area of loss (thick arrow). Some cells display vacuolated cytoplasm, faded basophilia (tailed arrow) and degenerated or fragmented nuclei (crossed arrow). The lumen of the tubules contains multiple vacuolations (V), and few or even no spermatozoa (double arrowhead). The widened interstitium shows homogenous acidophilic material (*), many vacuolations (arrow), dilated blood vessel (BV) and degenerated Leydig cells (arrowhead). 

C: **group (HA)** shows focal separation (wavy arrow) of the spermatogonia from the underlying basal lamina. The spermatogenic epithelium is formed of 2-4 rows, some spermatocytes have shrunken deeply stained nuclei (tailed arrow) others have fragmented nuclei (crossed arrow) and vacuolated deep acidophilic cytoplasm. The lumina of seminiferous tubules contain multiple vacuoles (V) and no spermatozoa (star). The Leydig cells (LC) appear normal. 

D: **group (HZ)** shows more improvement in the histological structure. Mildly congested blood capillaries (arrowhead) and apparently normal Leydig cells (LC) are demonstrated in the interstitium. The tubules are lined with 5-6 layers of spermatogenic cells with areas of loss (wavy arrow). The spermatogonia (SG), primary spermatocytes (Sp) and spermatids (Sd) and Sertoli cells (SC) have normal appearance. Spermatozoa (SZ) are noticed in the lumen of the tubules. 

E: **group (HAZ)** demonstrates evident enhancement in the histological structures of the testes more or less as group (C). The spermatogenic cells are arranged in multiple 7-8 rows and formed of normally appeared spermatogonia (SG), spermatocytes (Sp), spermatid (Sd) and multiple spermatozoa (SZ). Sertoli (SC) and Leydig cells (LC) also have normal appearance (H&E 400x, scale bar=25 μm).
Fig. 4: Representative photomicrograph of transverse section of the testes of various groups stained with MT. A: group (C) shows normal distribution of the collagenous fibers in the tunica albuginea (arrowhead) and around the walls (arrow) of the blood vessels (BV). B: group (H) shows noticeable increase in the collagenous fibers deposition in the tunica albuginea (arrow) and around the walls of the congested blood vessels (arrowhead). C: group (HA) demonstrates marked deposition of collagen fibers around severely congested blood vessels (arrowhead). D: group (HZ) shows more deposition of the collagenous fibers in the tunica (arrow) and around blood vessels (arrowhead). E: group (HAZ), normal distribution of the collagen fibers in the tunica albuginea (arrow) and around the wall of the blood vessels (arrowhead) is demonstrated (Masson's trichrome 200x, scale bar=50 μm).

Immunohistochemical Results:
The immunohistochemical assessment revealed that sections of the testes from group (H) (Fig. 5B) showed a weak positive PCNA immunoreactivity in comparison to group (C) (Fig. 5A). The immunostaining in group (HAZ) was strongly positive (Fig. 5E). Weaker reactivities were observed in both groups (HA and HZ) (Fig. 5C, D).
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Fig.5: Representative photomicrograph of sections of the testis of the different studied groups. Decreased PCNA immunoexpression (arrowhead in B) of group (H) compared to group (C) (arrowhead in A). Weaker immunoexpression is detected in groups (HA arrowhead in C & HZ arrowhead in D). However, group (HAZ) shows restoration of the immunoreaction (arrowhead in E) approaching that of the control. (PCNA Immunostaining 400x, scale bar=25 μm).

Digital Morphometric Assessment: Diameters and Perimeters of The Seminiferous Tubules:
Morphometric assessment of the testes of group (H) showed an insignificant decrease in the diameters of the seminiferous tubules when compared to group (C). In groups (HA, HZ and HAZ) the diameters were insignificantly increased in comparison with group (H) (Table 4 and Fig.6). On the other hand, the means of the perimeters of seminiferous tubules of group (H) exhibited significant decrease when compared to the group (C). However, the perimeters in the groups (HA, HZ and HAZ) were significantly increased in comparison with the group (H). Significant increases in the perimeters were also recorded in groups (HZ and HAZ) groups when compared with the groups (HA) (Table 4 and Fig.6).
Table 4: The mean diameters and perimeters of seminiferous tubules in different groups.

<table>
<thead>
<tr>
<th></th>
<th>Group C</th>
<th>Group H</th>
<th>Group HA</th>
<th>Group HZ</th>
<th>Group HAZ</th>
<th>P value</th>
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</thead>
<tbody>
<tr>
<td>Diameter</td>
<td>276.19±19.2</td>
<td>200.13±21.6</td>
<td>243.21±24.1</td>
<td>259.73±27.9</td>
<td>273.84±26.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Perimeter</td>
<td>879.03±21.7</td>
<td>643.69±58.2</td>
<td>762.33±78.9</td>
<td>828.001±91.2</td>
<td>875.57±88.6&lt;</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

\(a\): Significance vs group C. \(b\): Significance vs group H. \(c\): Significance vs group HZ. \(d\): Significance vs group HAZ.

Fig. 6: The mean diameters and perimeters of the seminiferous tubules in the studied groups.

Area Percentage of Masson Trichrome Staining:

The area percentage of MT staining in group (H) demonstrated a significant increase \((p<0.001)\) when compared to group (C). Moreover, the groups (HA, HZ, HAZ) showed a significant decrease in comparison with group (H) (Table 5 and Fig. 7).

Area Percentage of PCNA:

In group (H) the area percentage of the PCNA immunoexpression showed a significant decrease \((p<0.001)\) when compared with group (C). These percentages were significantly increased in groups (HA, HZ, HAZ) in comparison with group (H) (Table 5 and Fig. 7).

Table 5: Representing the area percentages of MT staining and PCNA immunoexpression.

<table>
<thead>
<tr>
<th></th>
<th>Group C</th>
<th>Group H</th>
<th>Group HA</th>
<th>Group HZ</th>
<th>Group HAZ</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Masson</td>
<td>3.62±0.7</td>
<td>11.32±1.2*</td>
<td>5.67±0.9*</td>
<td>4.73±0.9*</td>
<td>4.21±0.6* &lt; 0.001*</td>
<td></td>
</tr>
<tr>
<td>PCNA</td>
<td>9.88±1.7</td>
<td>1.8±0.2*</td>
<td>4.06±0.9*</td>
<td>6.99±1.2*</td>
<td>7.5±1.6* &lt; 0.001*</td>
<td></td>
</tr>
</tbody>
</table>

\(a\): Significance vs group C. \(b\): Significance vs group H. \(c\): Significance vs group HZ. \(d\): Significance vs group HAZ.
Atorvastatin and Zinc synergistically ameliorate High Fat Diet-Induced Testicular injury

**DISCUSSION**

Recently, the consumption of a hyperenergetic, highly palatable diet has been increasing in Western populations. This dietary pattern and sedentary lifestyle are significantly linked to the development of obesity (Gil-Cardoso et al., 2017). Over the past 40 years, obesity has been recognized as a global health challenge (Hruby and Hu, 2015) and its prevalence is increasing in adult males more than females, particularly in developed countries such as the UK, Australia and the USA (Ng et al., 2014). Obesity has a wide range of detrimental health effects, including an increased risk of type 2 diabetes, female malignancies (Foong and Bolton, 2017), dyslipidaemias, non-alcoholic fatty liver disease, and cardiovascular disorders (Johnson et al., 2015). Despite the advancement of human life, hyperlipidemia still poses a severe threat to human health and is relatively common. Statistics from the general population show that the incidence of hyperlipidemia has reached as high as 20–40% and is still rising (Kong et al., 2018). Enhanced triglyceridemia and free fatty acidemia are consequences of HFD-induced obesity (Rygiel, 2018). Increased ROS and/or inflammatory mediators caused by dyslipidemia are thought to be the main cause of metabolic diseases including infertility (Syriou et al., 2018). Moreover, obese males are more likely to develop azoospermia and other unfavorable semen parameters (Luque et al., 2017).

In the current experiment, the rats of group (H) showed a significant increase in total body weight when compared to control. Administration of AT and Zn together or separately significantly decreased the total body weight when compared to the group (H) similar to the results obtained by (Shalaby et al., 2004; Ommurugan et al., 2018). Additionally, the testicular weights were significantly decreased in group (H) when compared to group (C), several studies reported similar results (Luo et al., 2019; McPherson et al., 2019; Wang et al., 2019; McPherson and Lane, 2020). However, in the groups (HA, HZ and HAZ), the testicular weights exhibited a significant increase as compared to group (H) and this goes in accordance with (McPherson et al., 2019; Billah et al., 2022). Moreover, Zn administration could maintain and increase the weights of testes and other reproductive organs via the regulation of testosterone levels (Merrells et al., 2009; Reshma Anjum et al., 2017).

In this study, serum level of LH was significantly decreased in group (H) and by treating the animals with AT and Zn a significant increase in its level was detected. These results are like those

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**Fig. 7:** The mean area percentages of MT staining and PCNA immunoexpression in the different groups.
obtained by (El-Mehi and Faried, 2019). In addition, Serum levels of testosterone significantly decreased in group (H) when compared to group (C). Treating the rats with both AT and Zn resulted in a significant increase in testosterone levels. These findings go in consistent with (Vigueras-Villaseñor et al., 2011; Leite et al., 2014; Banihani, 2020). Zn exerts a crucial role in regulating the serum levels of testosterone (Rafique et al., 2009). Zn therapy increases serum testosterone levels (Saïd et al., 2010). Thus, the decline in testosterone levels may be assigned to abnormalities in the Leydig cells or reduced sensitivity to LH and/or direct suppression of androgen production in rats (Joshi et al., 2014). According to Dissanayake et al., (2009) and Joshi et al., (2014), dietary Zn shortage also decreased serum testosterone levels. Zn deficiency inhibits the pituitary gland's ability to release luteinizing and follicle-stimulating hormones, which promote the creation of testosterone. Additionally, Zn prevents the enzyme known as aromatase from turning excess testosterone into estrogen (Askary et al., 2011).

On the other hand, no differences in testosterone levels were detected in the results acquired by (Pons-Rejraji et al., 2014; Campos-Silva et al., 2015; De Oliveira et al., 2019; Abdel-Gabbar et al., 2020; Akdeniz et al., 2020). Additionally, Darbandi et al., (2018) explained that the formation of free radicals disrupts steriodogenesis by impairing the hypothalamus-pituitary-gonadal axis, and/or producing Leydig cell degeneration that ultimately results in a decrease in testosterone release (Li et al., 2019). These hormonal changes not only have negative impacts on the sexual act and spermatogenesis but also may clarify the degenerative alterations in the spermatogenetic epithelium (El-Mehi and Faried, 2019) since the normal architecture of the testis is testosterone-dependent (Das and Indira, 2015).

In this investigation, the SOD, CAT and GPX concentrations were significantly decreased in group (H) when compared to group (C). However, a significant increase in the MDA (a sensitive biomarker of lipid peroxidation) concentrations compared to the control rats was detected. Treating the group (H) with AT and Zn together resulted in a significant increase in SOD, CAT and GPX and a significant decrease in the MDA levels when compared to the group (H). This data goes with Chandramohan and Pari, (2014); El-Mehi and Faried, (2019) who confirmed a significant increase in MDA levels and a significant decrease of the SOD in homogenized testes of HFD rats, evidently indicating oxidative stress and compromised lipid peroxidation. According to Ozyurt et al., (2006); Shamsi et al., (2010) and Partyka et al., (2011), a positive correlation between sperm count and blood SOD can be established. This suggests that the SOD evaluation may serve as a biochemical parameter to measure sperm concentration as well as a marker of the oxidant environment around sperm.

The process of spermatogenesis, an active cell division, demands increased oxygen consumption in the mitochondria of the spermatogenic epithelium. Polyunsaturated fatty acids are abundant in testicular membranes, making them vulnerable to damage from lipid peroxidation (Asadi et al., 2017). Therefore, the testis contains an antioxidant defense mechanism that is made up of several enzymes like CAT, GPX, SOD, and nicotinamide adenine dinucleotide phosphate [NADPH] reductase. In addition to these enzymes, a number of tiny molecules, such as Zn, vitamin C, vitamin E, melatonin, and cytochrome C safeguard the testicles from oxidative damage (Aitken and Roman, 2008).

Zn deficiency raises lipid peroxidation and induces damage in rat tissues including the testis (Zhao et al., 2011) and consequently affecting the seminal parameters (Reshma Anjum et al., 2017). Shamsi et al., (2010) found that enhanced MDA levels are
concomitant to an increase in the percentages of dead and abnormal sperms. These results could be attributed to increased lipid peroxidation that can lead to loss of integrity and fluidity of the membrane, increased cellular permeability, DNA damage, enzyme inactivation, reduction of sperm fertilizing power and cell apoptosis. Zn is a crucial trace nutrient required for a variety of biological processes including the body's oxidative defense mechanism (Prasad, 2014). Zn increases the activity of antioxidant enzymes like SOD (Messaoudi et al., 2010). Since SOD is a Zn-dependent enzyme, Zn supplementation is anticipated to restore the enzyme activity. Zn functions as an antioxidant using two distinct methods. In the first mechanism, it guards sulfhydryl groups against oxidation. But in the second pathway, Zn prevents ROS formation (Jomova and Valko, 2011). Zn supplementation during diabetes retains and maintains GPX activity (Sahu et al., 2020). GPX is an abundant enzyme actively expressed in the mitochondria of spermatozoa and testes providing safe sperm transport and survival (Barranco et al., 2016). A substantial drop in GPX levels causes aberrant spermatozoa (Qazi et al., 2019). However, according to Sena-Evangelista et al., (2015), Zn supplementation did not restore the mineral status, lipid profile, GPX and SOD activity.

In the present work, histological examination of the testes of group (H) revealed disorganized, irregular and widely separated seminiferous tubules. Many tubules appeared atrophic with irregular and interrupted basal lamina. The spermatogenic epithelium showed several degenerative changes in the form of losses, reduction in their thickness, wide intercellular spaces and focal separation from the basal lamina. The germinal cells displayed vaculated cytoplasm and their nuclei showed variable changes such as fading of its basophilia, deep staining, shrinkage and fragmentation. Scarce spermatozoa, acidophilic hyalinized material and numerous vacuolations appeared in the lumina of the tubules. The interstitium appeared widened with multiple vacuolations, congestion of the capillaries and deposition of homogenous acidophilic material. Various interstitial cells of Leydig were degenerated and had shrunken deeply stained basophilic nuclei.

Atrophy of the seminiferous tubules may be manifested by intracellular cytoplasmic vacuolations (Sakr et al., 2003), and this may be due to lipid peroxidation-induced increase in the permeability of cell and organelles membranes. Widening of intercellular spaces among the spermatogenic cells as well as separation from the basement membrane were reported in previous studies Mohamed et al., (2014) and El-Mehi and Faried, (2019) who suggested that exposure to ROS disrupts the tight junctions of blood-testis barrier with the consequent intercellular influx of excess water and toxic agents. Moreover, increased glycerol levels due to HFD cause a leaky blood-testis barrier and impairment of the tubular fluid homeostasis with the subsequent promotion of germ cell apoptosis (Abdel-Gabbar et al., 2020). Another explanation was given by Anan et al., (2017) who reported that oxidative stress has a detrimental effect on Sertoli cells and their processes. Damage to the cellular processes of Sertoli cells results in the shedding and exfoliation of the spermatogenic cells into the lumen of the seminiferous tubules. The deposition of homogenous acidophilic material in the interstitium was also noted by Yousaf et al., (2016) and El-Mehi and Faried, (2019) who postulated that decreased serum testosterone levels may increase capillary filtration and substantial interstitial fluid. Moreover, accumulation of the induced ROS results in the enhancement of vascular permeability and excessive exudates from the degenerated lymphatics (Ravikumar and Srikumar, 2005; Lirdi et al., 2008).

In the present work, an improvement in the histological
architecture of the testes of group (HA) as compared to group (H) was observed. However, the thickening of the capsule and congestion of the blood vessels were still noticed. Also, within the seminiferous tubules, vacuolation, thinning of the lining spermatogenic cells and nuclear degenerative changes were demonstrated. The lumina of several tubules contained few spermatzoa. Moreover, minimal eosinophilic material in the interstitium was still present. These findings are in line with (Fraunfelder, 2004; Karam et al., 2008; Panonnummal et al., 2013), who ascribed that AT had a vasodilator impact. In addition, according to Undas et al., (2013), statins have been demonstrated to reduce vasoconstriction via elevating endothelial nitric oxide (NO) activity, which may be the reason for the congestion and vasodilatation effects of AT.

Atorvastatin (AT), the most prevalent synthetic statin, decreases plasma Levels of lipoproteins and cholesterol by inhibiting the enzyme HMG-CoA reductase (Gurel et al., 2019). Additionally, AT possesses advantageous biological effects, such as anti-inflammatory and antioxidant characteristics (Yang et al., 2017). AT have protective effects against toxicity in various tissues including the testis (Naeimi et al., 2017).

Moreover, the testicular section stained with MT in groups (H, HA, HZ) showed increased deposition of the collagen fibers in the capsule, wall of blood vessels and basal lamina. However, in HAZ rats, more or less normal distribution of the collagenous fibers was observed. HFD increases the production and release of some pro-inflammatory mediators such as prostaglandin E2 and tumor necrosis factor. These mediators, in addition to increasing ROS, induce inflammation and substantial fibrosis (De Oliveira et al., 2019). The above results indicate that AT has inflammatory and fibrotic effects and this coincides with previous studies (Lańcut et al., 2004; Westwood et al., 2005; Simsek Ozek et al., 2010; Pons-Rejraji et al., 2014). Despite being well-tolerated, statins trigger adverse effects such as muscle aches, elevated liver enzymes, and a higher risk of developing diabetes. Statins induce tissue damage by releasing several inflammatory cytokines that lead to the enhancement of mononuclear cellular infiltration (Mostafa et al., 2015). Also, Pal et al., (2015) described that AT administration increases lipid peroxidation by diminishing antioxidant enzyme activities and glutathione levels inducing nucleic acids and proteins impairment with subsequently increased collagen formation. Moreover, it negatively affects male reproductive tissues even though only a few experiments investigated this (Banihani, 2020). Recently, studies reported that statins raise sperm DNA fragmentation and reduce its motility (Leite et al., 2018). Furthermore, Statins cause germ cell apoptosis and alter sperm morphology through the impairment of lipid metabolism (Banihani, 2020). AT was reported to cause a significant deterioration in sperm development (Akdeniz et al., 2020). It also significantly decreased serum concentrations of LDL and TG. Decreased cholesterol concentrations induce a reduction in testosterone levels as all steroid hormones are formed from cholesterol (Hsieh and Huang, 2016). Testosterone is crucial for the transformation of spermatogonia A to spermatogonia B. Moreover, Testosterone is involved in spermatocytes and spermatids survival via enhancing anti-apoptotic mechanisms (Shiraishi and Matsuyama, 2017). Therefore, the testosterone levels decline may consequently cause impairment of spermatogenesis (Akdeniz et al., 2020). In addition, Siqueira et al., (2004) reported that decreased cholesterol levels lead to degeneration of the membranous organelles via modulating cell membrane fluidity and subsequent alteration in the Na/K pump functions. Furthermore, HMG-CoA
reductase inhibition deprives the cells of crucial antioxidant substances such as ubiquinone or coenzyme Q10 (Otruba et al., 2011). Also, statins were reported to have direct impacts on the mitochondrial respiratory chain that may lead to cell apoptosis (Otruba et al., 2011).

On the other hand, these findings were contrary to that obtained by (Mizuguchi et al., 2004; Barsante et al., 2005; Ou et al., 2008; Zhao et al., 2010) who demonstrated that statins alleviated drug-induced fibrosis in various tissues via decreasing collagen deposition. AT may alleviate HFD-induced poor fertility by improving dyslipidemia. The antioxidant action of AT, known as a pleiotropic effect, is thought to be the main mechanism by which it increases sperm parameters and aids in reversing fertility changes (Ommurugan et al., 2018). Additionally, AT helped in reducing vascular nitric oxide-derived and myeloperoxidase-derived oxidants. This activity was independent of the changes in statins-induced alterations on C-reactive protein and lipid profile (Shishehbor et al., 2003). Amelioration of the lipid profile fosters fatty acid oxidation, otherwise, fatty acids will be esterified, leading to the accumulation of TG and LDL (Brault et al., 2014). statins can share in this improvement by reducing CH biosynthesis and up-regulating the LDL receptor resulting in the clearance of LDL (Al-Naqeep et al., 2010).

In this work, the combination of Zn and AT decreased the collagen deposition, and this was confirmed morphometrically. Also, more improvement in the histological alterations was obviously detected. Zn promotes cell division and differentiation as it has a pivotal role in DNA replication, transcription and protein synthesis (Reshma Anjum et al., 2017). Zn supplementation was reported to maintain spermatogenesis (Fallah et al., 2018) and improve semen quality (Kumar et al., 2014) and sperm motility (Zhao et al., 2016) by reducing the apoptosis of the testicular and sperm cells. Moreover, Zn ameliorated the oxidative stress, damage to DNA and the subsequent cellular changes during spermatogenesis (Sahu et al., 2020).

We used the diameters and perimeters of the seminiferous tubules to evaluate the process of spermatogenesis. In the present work, group (H) showed an insignificant decrease in the diameters of the seminiferous tubules when compared to group (C). In groups (HA, HZ and HAZ) the diameters were insignificantly increased in comparison with group (H) and this goes inconsistent with (Campos-Silva et al., 2015). Moreover, we noticed significant decreases in the perimeters of the seminiferous tubules indicating their shrinkage. Similar results were reported by (Meydanli et al., 2018; El-Mehi and Faried, 2019).

In the current investigation, we employed immunohistochemistry to map the distribution of PCNA immunoreactivity in the testes of the various experimental groups. In group (H) the area percentage of PCNA immunostaining showed a highly significant decrease when compared with group (C). These percentages were significantly increased in groups (HA, HZ, HAZ) in comparison with group (H). The nuclear protein, PCNA, is necessary for DNA synthesis and has been widely used to identify the proliferating spermatogenic cells (Tousson et al., 2011). Previously, PCNA was used in the quantitative analysis of spermatogenesis, since the process of spermatogenesis is a complex cell cycle involving rapidly multiplying cells (Abdel-Dayem, 2009). Decreased area percentage of PCNA-positive immunostaining was also observed in a previous study (El-Mehi and Faried, 2019). XUE et al., (2007) related this to diminished DNA production and increased ROS-induced DNA breakdown which is commonly seen in infertility cases (Bennetts and Aitken, 2005).

This research hypothesized that the detrimental effects of HFD on the testis may be mediated via several mechanisms: elevated oxidative stress...
markers such as MDA and suppression of the antioxidants enzymes such as SOD, CAT and GPX. Additionally, disruption of steroidogenesis via impairing the hypothalamus-pituitary-gonadal axis. There is a great debate about the benefits and side effects of AT on the testis. Supplementation of Zn to AT in the treatment of dyslipidemia ameliorates testicular hazards of both AT and HFD.

**Conclusion:**

The findings of this study indicated that HFD exhibited deleterious functional and structural alterations in the testes of male albino rats. AT alone was proved to have controversial effects on the histological structure of the testes. These alterations could be attributed mainly to HFD ± AT. Accordingly, we highly recommended the concomitant administration of Zn with AT to alleviate these deleterious changes.

**Conflict of Interest:** The authors declare that they have no conflict of interest.

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**Author Contribution:** Ali EMT: Conceptualization, Methodology, Validation, writing original draft, Reviewing and Editing.

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Eyad M.T. Ali


ARABIC SUMMARY

يخفف مزيج الأتورفاستاتين والزنك من التغيرات البيوكيميائية والتنسجية التي تسببها النظام الغذائي عالي الدهون في خصية ذكور الجرذانبيضاء

أيد محمد طلبة علي

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قسم التشريح، كلية الطب، جامعة المنصورة، مصر.

يعتبر فرط الدهون الدم عاملاً حاسماً في التسبب في العقم. الأتورفاستاتين هو الخط الأول لعلاج اضطرابات الدهون في الدم والذي يمتاز بتاثيرات مضادة للالتهابات الأكسدة وكذلك قابلية للتصريفات. ومع ذلك، لوحظ انخفاض في جودة التستوستيرون والحيوانات المنوية. بعد الزنك عصرًا أساسيًا في نظام الدفع المؤكد، ونسخ الحمض النووي، وتوليد البروتين، وتكوين الحيوانات المنوية وهرمون الستيرويد. تعتبر هذه المعلومات مناقضة فيما يتعلق بتاثيرات الأتورفاستاتين على الخصية ومن ثم تهدف هذه الدراسة إلى تقييم الأثار الوقائية المحتملة للأتورفاستاتين والزنك منفصليًا أو مجتمعًا في إصابة الخصية التي يسببها النظام الغذائي عالي الدهون. أجريت الدراسة على أربعين ذكرًا من الجرذان البيضاء والتي تم توزيعها في خمس مجموعات: المجموعة الضابطة، المجموعة (H) التي تلقى نظام غذائي عالي الدهون، المجموعة (HA) تلقى نظام غذائي عالي الدهون مع الأتورفاستاتين، المجموعة (HZ)؛ تلقى نظام غذائي عالي الدهون مع الزنك، المجموعة (HAZ)؛ تلقى نظام غذائي عالي الدهون بالإضافة إلى الأتورفاستاتين والزنك لمدة ثمانية أسابيع. أدى استخدام الأتورفاستاتين والزنك معًا أو بشكل منفصل إلى تحسين أوزان الجسم والخصية بشكل ملحوظ مع التعافي الكبير في التقييم البيوكيميائي والهرموني. أظهرت النتائج في المجموعة (H) ضرورة ودعم انسياب النمو مع فقوع وقلة السمك وكذلك انفصال بؤري لتشنج المولد للحيوانات المنوية، وكذلك تدهور في الحيوانات المنوية مع وجود خبرات وقلة نتائج في الجرذان لإيجاد لفسخ ليحة (HAZ) و (HZ) في تحلق الأبنية، أما المجموعة (HAZ) فعندما تحلق في تحلق الأبنية. فعندما تحلق في تحلق الأبنية. وضمن ذلك، أدت النتائج إلى أن الأتورفاستاتين وحالياً بتأتي متضمنة للجزء على التركيب النسيجي للخصية، والتي يمكن أن تعرّف بشكل أساسي إلى تأثيرات الأتورفاستاتين والزنك على الأورفاستاتين للتخفيض من هذه التأثيرات الضارة.