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Effects of liraglutide on the testis of rats with experimental diabetes and ischemia-reperfusion injury

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ABSTRACT

Our study aimed to investigate the antidiabetic, antioxidative, and antiapoptotic effects of liraglutide on the testes of rats with diabetes and ischemia/reperfusion injury. Subjects were divided into three groups: control, diabetes, and torsion groups. Rats with diabetes were further divided into two subgroups such as diabetes and diabetes+Liraglutide groups. The torsion group was divided into three subgroups such as torsion, torsion/detorsion, and torsion/detorsion+Liraglutide groups. Malondialdehyde (MDA), superoxide dismutase (SOD), and testosterone levels were measured from blood samples. Also, testicular tissue samples were examined by light and electron microscopy. Apoptosis was assessed using immunohistochemistry for caspase-3. Degeneration of seminiferous tubules and interstitium was observed in the diabetes, torsion, and torsion/detorsion groups, while Liraglutide treated groups showed normal seminiferous tubules morphology. Elevated levels of apoptosis, i.e. caspase-3, were observed in diabetes, torsion, and torsion/detorsion groups ($P < 0.05$), whereas Liraglutide treated groups had similar levels of apoptosis as the control group. MDA levels of diabetes, torsion, and torsion/detorsion groups were increased ($P < 0.05$), while SOD and testosterone levels were decreased ($P < 0.05$). However, Liraglutide treated groups, SOD, MDA, and testosterone levels were found similar to the control group. In conclusion, Liraglutide positively affects structural changes and hormone levels in diabetes and torsion/detorsion groups.

KEYWORDS

Diabetes; GLP-1 analog; liraglutide; testis; torsion

Introduction

Damage to the testes by testicular torsion and indirectly by diabetes mellitus can significantly impact male fertility. Testicular torsion is an important health problem that occurs with the rotation of the spermatic cord around itself (Zhao et al. 2011). The main pathology in torsion, which causes a decrease in blood flow, are biochemical and morphological changes that occur with tissue damage caused by free oxygen radicals (ROS) following torsion (Filho et al. 2004; Sharp et al. 2013). Diabetes Mellitus (DM) is a systemic disease that develops due to increased blood glucose levels as a result of insulin deficiency and insulin resistance or a combination of both, which can cause chronic and acute complications. Diabetes Mellitus can cause spermatogenic cell damage, erectile dysfunction, decrease in sperm motility and semen volume, and it has been suggested that it significantly affects male fertility directly and/or indirectly (Agbaje et al. 2007; Kasturi et al. 2008; He et al. 2021). Testicular hypoxia is characterized by insufficient oxygen within the testicular microenvironment, triggers oxidative stress, dysregulated apoptosis, and impaired spermatogenesis, ultimately culminating in testicular injury, male infertility, and reproductive dysfunction (Shokoohi et al. 2024).

Liraglutide is a glucagon-like peptide-1 (GLP-1) agonist (Knudsen et al. 2000) that provides significant glycemic control and reduces the risk of hypoglycemia by reducing body weight in adult T2D patients (Buse et al. 2009; Chitnis et al. 2014). While it has extrapancreatic effects (Kalra and Gupta 2016), there are very few studies that assess the effect of Liraglutide on various organs such as heart, lungs, kidneys, and brain, on the male reproductive system (Chitnis et al. 2014). In addition to its weight loss effect, Liraglutide can increase testosterone levels and sperm counts and decrease the sperm abnormality rate of obese male mice (Qi et al. 2024). Liraglutide, whose antidiabetic effect is widely accepted in clinical and experimental studies, has also been reported to have antioxidative and antiapoptotic effects (Inoue et al. 2015; Yu et al. 2023). This study aimed to investigate the antidiabetic, antioxidative, and antiapoptotic effects of Liraglutide in testis by light and electron microscopic, immunohistochemical, and biochemical methods in experimentally induced diabetes and torsion/detorsion models which caused different tissue damage from each other.

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Materials and methods

The experimental procedure

In our study, 48 adult male Wistar albino rats with an average weight of 200–300 g were used. Experimental animals were kept in Cukurova University Experimental Medicine Research and Application Center conditions: in a room with an average temperature of 22°C, subjects were kept in 12 h of light and 12 h of darkness in plastic cages with stainless wire covers. Pellet feed and tap water were given to the animals ad libitum. Thirty-six subjects were divided into three groups: diabetes, torsion, and control groups. The number of subjects in the groups was determined as a result of the statistical power analysis.

Group 1 (Control Group) n = 8	Group 2 (Diabetes Group) n = 16	Group 3 (Torsion Group) n = 24
	Group 2a (Diabetes) n = 8 Group 2b (Diabetes +Liraglutide) n = 8	Group 3a (Torsion) n = 8 Group 3b (Torsion/Detorsion) n = 8 Group 3c (Torsion/Detorsion +Liraglutide) n = 8

Group 1: Control group (n = 8): No application procedure was done.

Group 2: Diabetes group (n = 16): 50 mg/kg Streptozocin (SO130, Sigma) dissolved in 0.01 M sodium citrate buffer (pH 4.3) was administered intraperitoneally to the subjects who were fasted the day before. After 72 h, blood samples taken from the tail vein were measured with a glucometer, and those with blood glucose levels >300 mg/dL were considered diabetic and included in the study. The diabetes group comprised 16 subjects divided into two subgroups: the non-treated diabetes and the Liraglutide treated groups.

Group 2a. Diabetes Group (n = 8): Subjects were kept alive under normal laboratory conditions for 14 days.

Group 2b. Diabetes + Liraglutide (n = 8): Liraglutide (Nova Nordisk, USA) at a dose of 0.3 mg/kg was administered subcutaneously twice a day for 14 days.

Group 3: Torsion group (n = 24): Torsion was performed by rotating the left testicles of the subjects under anesthesia by 720° clockwise. Torsioned subjects were evaluated in three subgroups such as the non-treated torsion group, the non-treated torsion/detorsion group and the Liraglutide treated group.

Group 3a. Torsion group (n = 8): It was sacrificed after a 3-h torsion period.

Group 3b. Torsion/Detorsion Group (n = 8): Detorsion was performed after 3 h of torsion. Subjects with reperfusion were kept alive under normal laboratory conditions for 14 days.

Group 3c. Torsion/Detorsion+Liraglutide (n = 8): In the second hour of torsion, 0.3 mg/kg Liraglutide was injected subcutaneously, 1 h after the detorsion was performed and the injection was continued for 14 days, twice a day.

Light microscopic methods

To perform light microscopic examinations, one of each animal's testes was fixed in Bouin's solution for 8 h and dehydrated for 6 h with 50% alcohol and then with 70% alcohol. Tissue samples were washed under tap water and then dehydrated with an alcohol series (70%, 80%, 90%, 96%, and 100%). Thereafter, the tissues were kept in xylene for 1 h in three separate containers at the same concentration. After xylene treatment, tissues were kept in molten paraffin at 60°C which was allowed to solidify at room temperature overnight. Paraffin-embedded tissue sections were cut by a microtome (Shandon Finesse 325, Thermo Scientific) to a thickness of 5 µm. After staining with hematoxylin and eosin, tissue sections were examined under a light microscope (Olympus BX53, Tokyo, Japan) (Coşkun et al. 2016).

Morphometric methods

Testis sections from each group were evaluated for structural changes according to tubular diameter and epithelial height. Epithelial height and diamter of the seminiferous tubular wall was measured quantitatively by light microscopy (20× magnification). In each section, 10 randomly chosen circular cross sections of seminiferous tubules were measured by an Image J program.

Immunohistochemical methods

After routine tissue attachments with a Leica TP 1020 auto technical device, paraffin sections with a thickness of 4 µm were taken from the tissue specimens embedded in paraffin. After being dewaxed in xylene and rehydrated in graded alcohol, 4 µm thick paraffin sections were treated with heat-induced epitope retrieval solution and put into microwave irradiation for 10 min. Then, the sections were cooled off, washed in PBS, and incubated with 3% H_2O_2 to block endogenous peroxidase for 15 min at room temperature. After washing three times in PBS, the sections were blocked with blocking (IHC Kit, TL-125-HD, Thermo Scientific) for 15 min. The primary antibodies including anti-caspase-3 (1:500; anti-rat rabbit polyclonal antibody, ab4051, Abcam, MA, USA) antibodies were added and the samples were incubated overnight at 4°C. Negative control slides were prepared by omitting the primary antibodies. After washing them on the next day, the secondary antibody

(IHC Kit, TL-125-HD, Thermo Scientific) was added which was followed by staining with 3-amino-9- ethylcarbazole (AEC IHC Kit ab93705, Abcam, MA, USA) for 10 min and hematoxylin reagent for 3 min before dehydration. Images of the stained sections were analyzed and photographed by light microscopy (Olympus BX53 Tokyo, Japan). To investigate the number of caspase-3 positive cells, an immunohistochemical scoring method was used. Five areas were picked at random at 20 times magnification for H-score assessment of each antibody in sections of the testis. The positive cells were enumerated visually by four individuals and the mean values were calculated (Bolat et al. 2016).

Electron microscopic methods

For electron microscopy, the other testis of each animal were fixed in 5% glutaraldehyde in a phosphate buffer (pH 7.2) for 24 h before tissue pieces were postfixed in 1% osmium tetroxide. Thereafter, the tissue was dehydrated in graded ethanol, embedded in Araldite, and processed for electron microscopy. The stained sections were examined with a JEOL-JEM 1400 transmission electron microscope (Coskun et al. 2019).

Biochemical methods

Intracardiac blood samples were centrifuged at 3600 rpm for 5 min to obtain serum. The serum was kept in Eppendorf tubes at −20°C. Serum SOD, MDA, and testosterone levels were, respectively, measured through SOD (E-EL-R1424, Elabscience), MDA (E-EL-0060, Elabscience), and testosterone (CSB-E05100r, Cusabio), ELISA kits in the ELISA Reader (Epoch) at the Medical Biochemistry Department of Cukurova University. SOD activity was measured in serum samples using the Cytochrome c reduction method. Serum MDA levels were determined using the TBA (Thiobarbituric Acid) reagent method.

Results

Light microscopic results

In the light microscopic examination of testicular tissue samples of the control group subjects, the seminiferous tubules and interstitium were intact (Figure 1, 1A–1B). In the diabetes group, severe vacuolization or immature cell accumulation in the lumen was observed in many seminiferous tubules surrounded by irregular membrane propria. In addition, edematous areas in the interstitium were noted (Figure 1, 2A–2B). In the diabetes+Liraglutide group, relatively normal seminiferous tubules and mild edematous interstitium were observed. Membrane propria was more regular than the diabetes group. However, it was determined that immature cell accumulation continued in some tubules (Figure 1, 3A–3B). In the testicular tissue sections of the torsion group, shrinkage of the seminiferous tubules and complete shedding of the tubule epithelium were observed. Multinucleated giant cells and apoptotic bodies were observed in the lumen of the tubules. The interstitium was edematous and hemorrhagic (Figure 1, 4A–4B). In the Torsion/Detorsion group, although some seminiferous tubules were similar to the Torsion group, spermatogenic cells were present in some tubules. In addition to immature cell accumulation, multinucleated giant cells and apoptotic bodies were observed in the tubule lumen. Diffuse edema and inflammation were detected in the interstitium (Figure 1, 5A–5B). In the Torsion/Detorsion+Liraglutide group, the presence of relatively normal seminiferous tubules was noted. Spermatogenic cells and Sertoli cells were observed to be morphologically normal in most seminiferous tubules, while mild edema was observed in the interstitium (Figure 1, 6A–6B).

Morphometric results

The ubular diameter and epithelial height of each group are shown in Table 1. The diabetes group, torsion group, and torsion/detorsion group had significantly lower ($P < 0.05$) tubular diameter and epithelial height when compared with the control group. However, there were no significant differences ($P = 0.33$) between the control group and the Liraglutide treatment groups.

Immunohistochemistry results

While minimal caspase-3 expression was observed in testicular sections of the control group (Figure 2A), intense expression was seen in some tubules, especially in spermatids in the post-meiotic stage, in the diabetes group (Figure 2B). Also, caspase-3 expression in the diabetes+Liraglutide group was similar to the control group (Figure 2C). In the torsion group, caspase-3 expression was observed especially in spermatids (Figure 2D). Caspase-3 expression was noted in both spermatids and Leydig cells in many tubules in testicular sections belonging to the Torsion/Detorsion group (Figure 2E). In addition, in the Torsion/Detorsion + Liraglutide group, caspase-3 expression was seen to decrease in many tubules and continue in Leydig cells especially (Figure 2F). Caspase-3 positive cells were seen to increase significantly in the diabetes group, torsion, and Torsion/Detorsion groups.

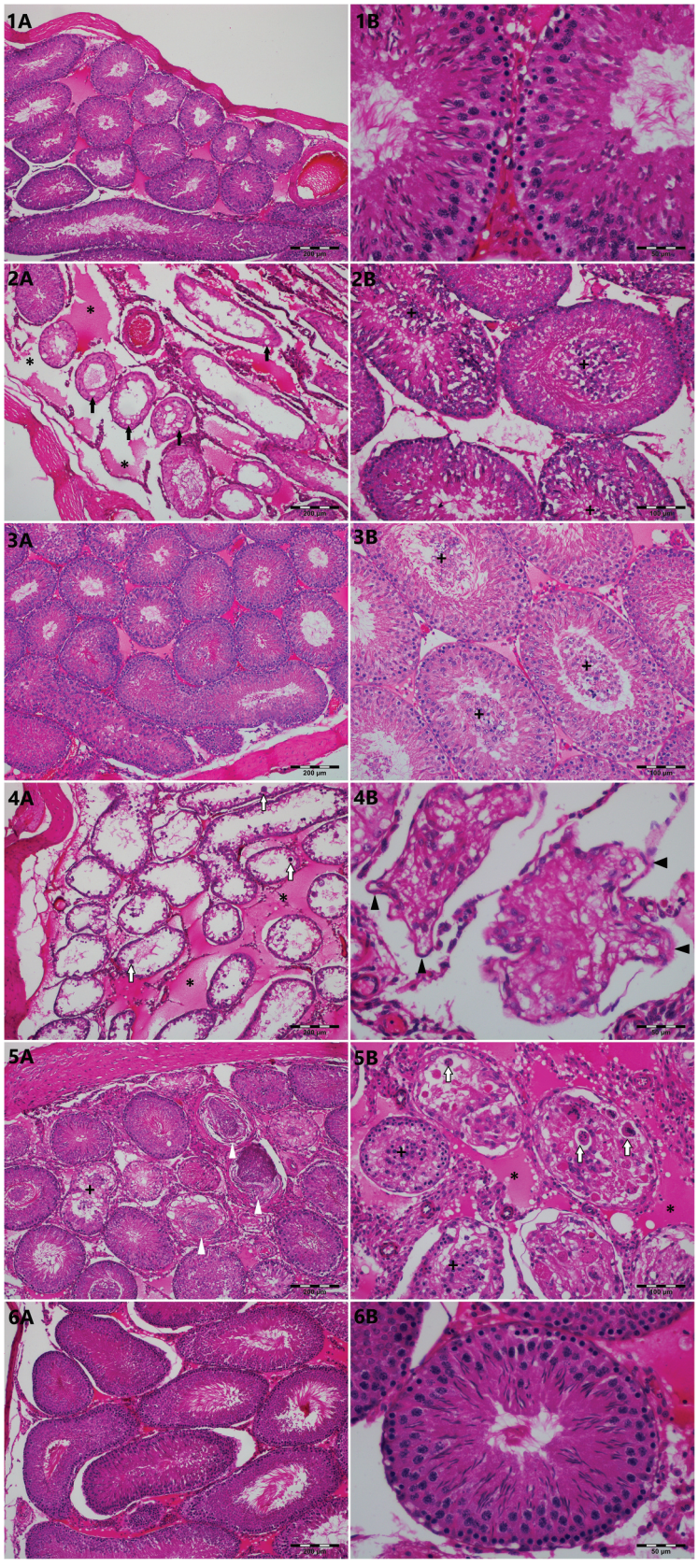

Figure 1. Light microscopic examination of the testicular tissue samples. **1A–1B**: Seminiferous tubules and interstitial area were observed to be normal in the control group, **Barr: 200 µm–100 µm. 2A–2B**: There was vacuolization (black arrow) in the seminiferous tubule epithelium, immature cell accumulation (+) in tubules luman, and interstitial edema (*) in the diabetes group, **Barr: 200 µm–100 µm. 3A–3B**: Immature cell accumulation (+) was observed in few seminiferous tubules of the

Table 1. Mean values of the morphometric parameters.

Mean		Tubular diameter (µm)	Epithelial height (µm)
Group 1	C	252.34 ± 33.15	81.34 ± 13.22
Group 2	D	208.16 ± 25.39[a]	43.81 ± 9.56[a]
	D + L	237.67 ± 43.24[b]	65.68 ± 11.46[b]
Group 3	T	196.12 ± 23.18[a]	34.54 ± 8.38[a]
	T/D	212.87 ± 39.66[a]	45.79 ± 7.56[a]
	T/D + L	229.11 ± 28.78[b]	63.14 ± 10.69[b]

[a]P < 0.05, significant compared to control.
[b]P > 0.05, non-significant compared to control.

Also, a significant decrease in the number of caspase-3 positive cells were observed in Liraglutide-treated groups when compared to the diabetes group, torsion, and Torsion/Detorsion groups (Figure 2G).

Electron microscopic results

When the testicular tissue samples of the control group were evaluated at the ultrastructural level, it was observed that the seminiferous tubules were surrounded by normal membrane propria. Sertoli cells and spermatogenic cells, Leydig cells in the interstitium, fibroblasts, macrophages, mast cells, blood, and lymph vessels were intact (Figure 3, 1A–1B). In the diabetes group, lysosomal structures, giant lipid droplets, SER vacuolization, and swollen mitochondria were observed in the cytoplasm of electron-dense Sertoli cells. However, the tight junctions between the Sertoli cell and the Sertoli cell were normal. The spermatogonia were shrinkage and irregular spaces formed around them. Spermatocytes had irregular nuclear membranes and swollen mitochondria in their cytoplasm. In some tubules, occasional disruptions in the acrosomal sheath were noted in spermatids in the acrosomal phase. Mitochondrial degeneration and SER vacuolization were also observed in the cytoplasm of Leydig cells (Figure 3, 2A–2B).

In the Diabetes+Liraglutide group, electron density in Sertoli cells continued, and mild SER vacuolization as well as increased lipid droplets of different sizes and lysosomal structures were observed. While the shrinkage continued in the spermatogonia, the spermatocytes were in normal structure reflecting the spermatogenesis process. The nucleus contour was irregular, and there were occasional dissolutions in the nuclear sheath. In addition to immature cells, phagocytic and apoptotic bodies were also present in the tubule lümen. In the Leydig cell cytoplasm, it was observed that lipid droplets and SER vacuolization decreased, but mitochondrial degeneration continued (Figure 3, 3A–3B). In the torsion group, the cellular integrity of Sertoli cells and the tight connections between them were disrupted, and the presence of giant lipid droplets, degenerative mitochondria, and dense structures, as well as extensive SER vacuolization in their cytoplasm were observed. Spermatogenic cells exhibited apoptotic and lytic changes. Hemorrhagic areas were noted in the tubule epithelium. While some Leydig cells became electron-dense, some exhibited lytic changes. Their cytoplasm contained SER vacuolization and many degenerated mitochondria (Figure 3, 4A–4B).

In the Torsion/Detorsion group, the total thickness of the membrane propria was extremely increased. The basal lamina was bilaminar, convoluted and irregular. Widespread SER vacuolization, degenerative mitochondria, as well as giant lipid droplets, were observed in the cytoplasm of Sertoli cells. However, tight junctions between Sertoli cells were still present in some tubules. Immature abnormal spermatids and apoptotic bodies were found in the tubule lumen. Some Leydig cells had increased electron density and degenerative mitochondria were seen in their cytoplasm (Figure 3, 5A–5B). Membrana propria of the Torsion/Detorsion+Liraglutide group was similar to the control group. The tubule structure was relatively similar to the control group, except that SER vacuolization was observed in the cytoplasm of some Sertoli cells, as well as spermatids with double nucleoli or dissolution of the nuclear membrane. Leydig cells containing diffuse SER, tubular crystalline mitochondria, advanced Golgi complex, lipid droplets, and lysosomal structures were also normal (Figure 3, 6A–6B).

diabetes+liraglutide group, **Barr: 200 µm–100 µm. 4A–4B**: Multinuclear giant cells (white arrow) were seen in almost all degenerative seminiferous tubules, also irregular contours of the seminiferous tubules (black arrow) and interstitial edema (*) were observed in the torsion group, **Barr: 200 µm–50 µm. 5A–5B**: Many degenerative seminiferous tubules (white arrowhead), also multinuclear giant cells (white arrow), immature cell accumulation (+), and interstitial edema (*) were seen in the torsion/detorsion group, **Barr: 200 µm–100 µm. 6A–6B**: Seminiferous tubules and interstitial area were in the normal structure in torsion+liraglutide group, **Barr: 200 µm–50 µm**.

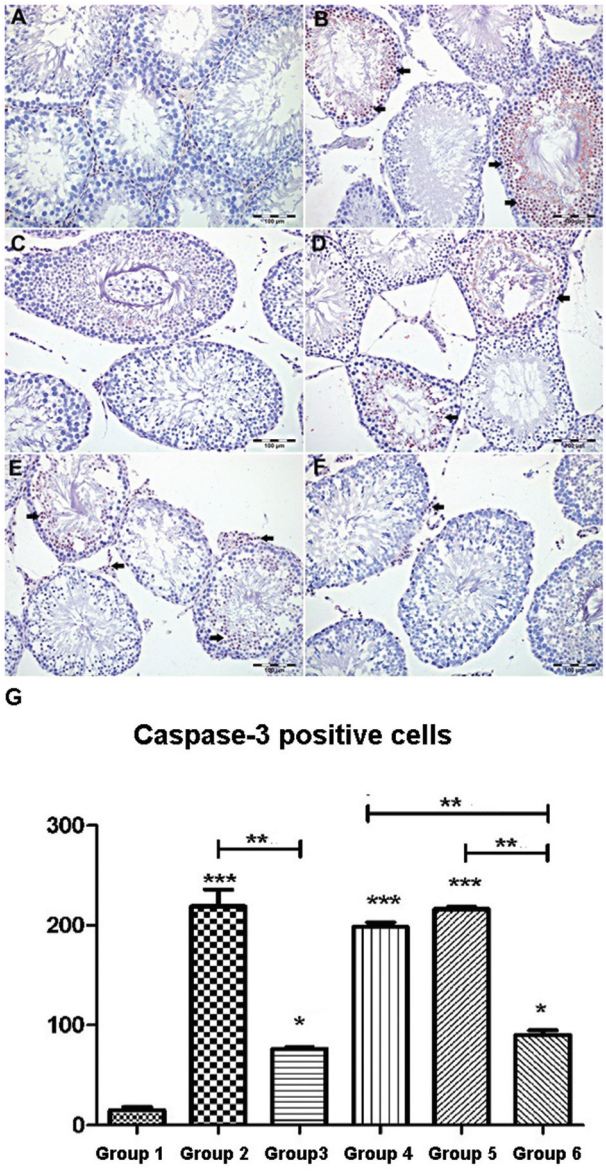

Figure 2. Caspase-3 immunoreactivity in testicular tissue samples of subjects. **A**: Very low Caspase-3 expression was observed in spermatogenic cells and Leydig cells in testicular tissues of the control group, **Barr: 100 µm. B**: Caspase-3 expression increased especially in spermatids on testicular tissues of the diyabetic group, **Barr: 100 µm. C**: Minimal Caspase-3 expression was seen in testiscular tissues of the diyabetic+Liraglutid group, **Barr: 100 µm. D**: Intensive Caspase-3 expression was observed especially in spermatids on testicular tissues of torsion group, **Barr: 100µm. E**: High Caspase-3 expression was seen in spermatids and Leydig cells on testicular tissues of the torsion+detorsion group, **Barr: 100 µm. F**: Caspase-3 expression decreased in spermatids, while contuening in Leydig cells in testicular tissues of torsion +liraglutid group, **Barr: 100 µm**.

Biochemical results

A significant increase was observed in MDA values of the diabetes, torsion, and torsion/detorsion groups ($P < 0.05$), but no significant difference was seen in the diabetes +Liraglutide and torsion/detorsion+Liraglutide groups ($P > 0.05$) when compared with the control group. Likewise, when SOD values were examined, a significant decrease was observed in the diabetes, torsion, and torsion/detorsion groups ($P < 0.05$), while no significant difference was observed in the diabetes+Liraglutide and torsion/detorsion+Liraglutide groups when compared to the control group ($P > 0.05$) (Table 2). When the testosterone values were compared with the control group, it was observed that the testosterone level in the diabetes, torsion, and torsion/detorsion groups was low, and it increased significantly in the diabetes+Liraglutide and torsion/detorsion+Liraglutide groups ($P < 0.05$) (Table 3).

Discussion

Diabetes is a systemic disease that can cause acute and chronic complications. If hyperglycemia is not controlled in this disease, the risk of morbidity and early mortality increases. Degenerative changes in various tissues and organs have been reported in many studies (Kakimoto et al. 2002; Kilarkaje and Al-Bader 2015; Sadi et al. 2015). In recent years, diabetes has been considered one of the causes of infertility in men (Jain and Jangir 2014). It causes oxidative stress by affecting many signaling pathways and causes structural and functional disorders in male reproductive system organs.

In our study, vacuolization in the tubule epithelium and immature cell accumulation in the tubule lumen were noted at the light microscopic level in the diabetes groups. It was observed that, at the ultrastructural level, Sertoli cells became electron-dense and contained giant lipid droplets and SER vacuolization in their cytoplasm. In addition, irregularities in the acrosomal sheath were observed in spermatogenic cells, especially in acrosomal phase spermatids. Studies have reported degenerative changes in membrane propria and seminiferous tubule epithelial cells, supporting our findings in I/R injury (Kaya and Harrison 1975; Dokmeci et al. 2007). It was observed that our light and electron microscopic results in the torsion/detorsion+Liraglutide group were similar to the control group. According to these results, it can be concluded that Liraglutide has minimal positive effects on testicular structure. However, it can be suggested that Liraglutide may have a feature that triggers the formation of multinucleated giant cells. Also, significant increase in tubular diameter and epithelial height was observed in the torsion/detorsion +Liraglutide group when compare to torsion and torsion/detorsion groups.

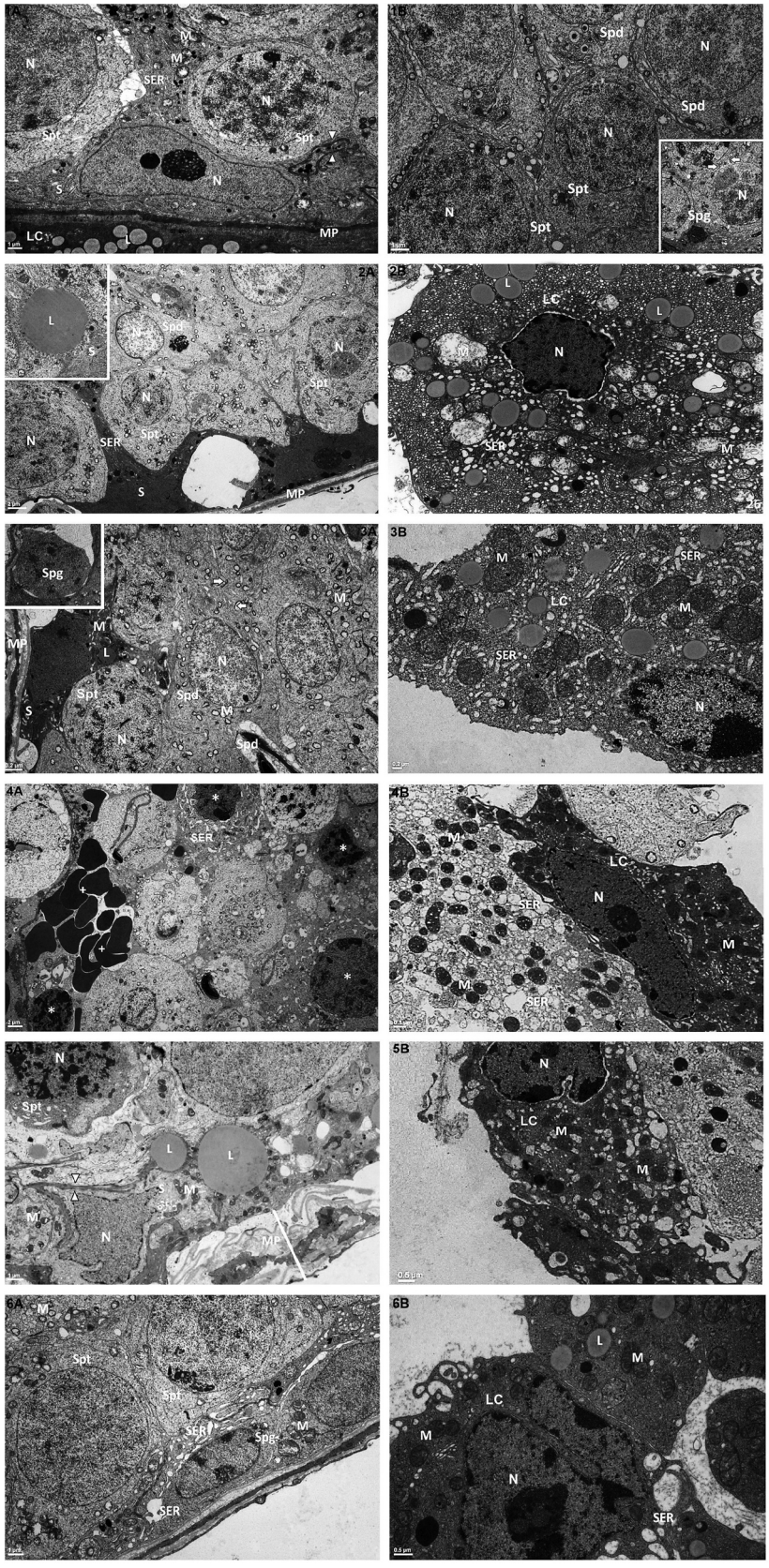

Figure 3. Electron microscopic view of testicular tissue samples. **1A**: Membrane propria (MP), Sertoli cells (S), spermatocytes (Spt) and nucleus (N) were seen to be normal in the control group. Tight connections between the Sertoli cells (arrowhead) were observed as normal like mitochondria (M) and agranular endoplasmic reticulum (SER). Leydig cells (LC) were seen to have normal abundant lipid droplets (L) in their cytoplasm, **Barr: 1 µm. 1B:** Nucleus (N) of spermatogonia (Spg), spermatocytes (Spt), and spermatids (Spd)

Table 2. Mean values of serum MDA and SOD.

Mean		Tubular diameter (μm)	Epithelial height (μm)
Group 1	C	5.2 ± 2.7	74.9 ± 6.9
Group 2	D	10.72 ± 2.4[a]	62.7 ± 5.4[a]
	D + L	7.13 ± 2.2[b]	69.3 ± 3.3[b]
Group 3	T	8.3 ± 1.17[a]	68.2 ± 3.4[a]
	T/D	10.23 ± 2.2[a]	64.8 ± 3.3[a]
	T/D + L	6.98 ± 0.12[b]	70.1 ± 4.02[b]

[a]P < 0.05, significant compared to control.
[b]P > 0.05, non-significant compared to control.

Table 3. Mean values of serum testosterone.

Mean		Testosterone (ng/ml)
Group 1	C	11.7 ± 1.12
Group 2	D	4.7 ± 0.2[a]
	D + L	5.2 ± 1.8[a]
Group 3	T	4.3 ± 1.2[a]
	T/D	3.8 ± 1.2[a]
	T/D + L	8.8 ± 0.2[a]

[a]P < 0.05, significant compared to control.

Liraglutide is an agonist of GLP-1, which is widely used in treating diabetes. It has also been studied in the cerebral ischemia model and has been suggested to increase the expression of the anti-apoptotic genes Bcl-2 and Bcl-xl (Briyal et al. 2014). In our study, a significant increase in caspase-3 expression was observed in the diabetes and torsion/detorsion groups. In contrast, a significant decrease was seen in the treatment groups as a result of the anti-apoptotic effect of Liraglutide in the testis, similar to literature. Our results about the antiapoptotic properties of Liraglutide were similar to reports of Değirmentepe et al., which determined the moderate anti-inflammatory, antioxidant, and antiapoptotic effects of Liraglutide against I/R injury in testicular torsion (Degirmentepe et al. 2021).

In conclusion, the antidiabetic, antioxidative, and antiapoptotic effects of Liraglutide, a GLP-1 agonist, on testis in experimental diabetes and torsion/detorsion model in rats were investigated. It was decided that the positive effects of this agent on the structural changes and hormone levels in diabetes and torsion/detorsion groups may be more effective in long-term use than twice in 14 days. It has been concluded that Liraglutide may be useful in clinics as an alternative treatment agent for preserving testicular structure and function in diabetic patients and/or torsion/detorsion cases. In the future, with further investigation into the molecular effective mechanism of Liraglutide, other oxidative stress-caused diseases can be treated.

were observed normal in control group, **Barr: 1μm. 2A**: Giant lipid droplets (L) (insert) and giant spaces were seen in the cytoplasm of electron dens Sertoli cells (S) in the diabetic group. Irregularity in the nuclear membrane of spermatocytes (Spt), and abnormally oriented spermatids (Spd) were also observed. Nucleus (N), Agranular endoplasmic reticulum (SER), Membrana propria (MP), **Barr: 2 μm. 2B:** Agranular endoplasmic reticulum (SER) vacuolization, degenerative swollen mitochondria (M), and decrease in lipid droplet (L) were seen in the cytoplasm of Leydig cells (LC) in the diabetic group, **Barr: 0.2 μm. 3A:** Giant spaces between electron-dense Sertoli cells (S) and shrikaged spermatogonia (Spg) (insert) were seen in the diabetes+Liraglutide group. Spermatocytes (Spt), spermatids (Spd), and cytoplasmic bridges (arrows) between spermatogenic cells were observed normal. Mitochondria (M), **Barr: 2 μm. 3B**: Except for agranular endoplasmic reticulum (SER) vacuolizatioan, Leydig cells (LC) were observed to have normal mitochondria (M) and lipid droplets (L) in the diabetes+Liraglutide group, **Barr: 0.2 μm. 4A:** Hemorrhagic areas (+) and pycnotic and apoptotic (*) spermatogenic cells are observed in the torsion group, **Barr: 2 μm. 4B**: Degenerative mitochondria (M) and vacuolized agranular endoplasmic reticulum (SER) were observed in Leydig cells (LC), which showed different electron density, in the torsion group, **Barr: 0.5 μm. 5A**: Increase in total thickness of membrane propria (MP) and giant lipid droplets (L) in the cytoplasm of Sertoli cell (S) were observed in the torsion+detorsion group. Nucleus (N), Mitochondria (M), and Tight junctions (arrowhead), **Barr: 1 μm. 5B**: Degenerative mitochondria (M) are observed in Leydig cells (LC) with different electron density, in the torsion +detorsion group, **Barr: 0.5 μm. 6A**: Normal electron densitive Sertoli cell (S) was observed in the torsion+liraglutid group. Membrana propria (MP), Nucleus (N), Mitochondria (M), and Tight junctions (arrowhead), **Barr: 1μm. 6B**: Leydig cell (LH) at normal electron density was observed in the torsion+liraglutid group. Nucleus (N), Mitochondria (M), Lipid droplet (L), and Agranular endoplasmic reticulum (SER), **Barr: 0.5 μm**.

Abbreviations

ROS, Free oxygen radicals; SOD, Superoxide dismutase; GSH, Glutathione; MDA, Malondialdehyde; GLP-1, Glucagon-like peptide-1; SER, Smooth endoplasmic reticulum; STZ, Streptozotocin; T1D, Type 1 diabetes; T2D, Type 2 diabetes.

Authors contributions

Performed experiments, analyzed the data, and wrote the manuscript: SH and GC. Supervised the work and revised the manuscript: HÖ, LS, AT, and EDY. Overall managed/organized the research work, conceptualized the study design, finalized the manuscript, and managed the manuscript submission: GC and SP. All authors contributed to the article and approved the submitted version.

Disclosure statement

The authors declare no conflict of interest.

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Ethical approval

All experimental procedures and tissue sampling were performed with the approval of the local ethics committee for animal experiments (25.02.2015) from Çukurova University Health Sciences Experimental Medicine Research and Application Center.

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