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Protective effect of liraglutide on experimental testicular ischaemia reperfusion in rats

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Abstract

This study was performed to evaluate the effect of liraglutide on experimental testicular ischaemia reperfusion in rats in terms of biochemistry, histopathology and immunohistochemistry. A total of 28 male Wistar-Albino rats were divided randomly into 4 groups: control (7), sham (7), ischaemia-reperfusion (7) and ischaemia-reperfusion + liraglutide (7). Biochemically, Nitric Oxide, Malondialdehyde, Superoxide dismutase, Glutathione peroxidase and Catalase levels were measured in the testis. Apoptosis protease activating factor-1 and inducible nitric oxide synthase activity were evaluated immunohistochemically as well. Statistical analyses were made via the Kruskal–Wallis and Mann–Whitney U tests. In the reperfusion group, CAT and SOD values were increased ($p > .05$), NO and MDA values were decreased ($p < .05$) after administration of liraglutide. In addition, GPx values were significantly increased in ischaemia reperfusion + liraglutide administered group compared to reperfusion group ($p < .05$). Apaf-1 and iNOS activity were significantly decreased with the addition of liraglutide treatment to the ischaemia-reperfusion group ($p < .05$). First of all, we would like to say that liraglutide treatment is moderately preventive against I/R injury in testicular torsion. The anti-inflammatory, antioxidant and antiapoptotic properties of liraglutide are create a moderately protective effect as we show in this study.

KEYWORDS

ischaemia–reperfusion, liraglutide, oxidative stress, testicular torsion

1 | INTRODUCTION

One of the common surgical emergencies in neonates, children and adolescents is testicular torsion. The prevalence of testicular torsion in men under 25 is 1/4000. After the rotation of the spermatic cord, the blood flow of the testicle is disrupted, which causes testicular damage. The testicular injury after torsion varies depending on both duration and degree of rotation (Moghimian et al., 2016). To ensure blood flow, rapid diagnosis and surgical detorsion is required in all cases to protect the testis tissue and prevention of orchiectomy. In spite of an effective surgical intervention, infertility or testicular atrophy can develop in 40–60% of the patients (Filho et al., 2004).

Although the first pathology is tissue ischaemia, ischaemia/reperfusion (I/R) injury subsequent to testicular torsion detorsion contributes to the damage (Yildirim et al., 2018). Some changes show up in the histopathology, biochemistry and blood components of the testis in reaction to ischaemia and reperfusion. Restoring the flow of blood to the ipsilateral testes after ischaemia results in overproduction cytokines, adhesion molecules and free oxygen radicals (FORs), and additionally, the migration of leukocytes. The base

pathophysiology of testicular torsion is surgical detorsion causes reperfusion injury due to FORs (Anim et al., 2005).

The Glucagon-like peptide-1 (GLP-1) analog liraglutide has been reported to reduce high glucose-induced oxidative stress, tumour necrosis factor (TNF) −α and inflammatory reaction in human endothelial cells (Shiraki et al., 2012). Inhibition of protein kinase C (PKC) translocation, nuclear factor (NF) -B activation and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activation contribute to the anti-inflammatory and anti-oxidative effects of liraglutide (Batchuluun et al., 2014). Additionally, liraglutide reduces the level of plasma reactive oxygen metabolites, which an indicator of oxidative stress in patients with type 2 diabetes (Okada et al., 2014). Liraglutide has been reported to reduce myocardial I/R damage in a recent study (Cheng, Wang, & Teng, 2015). In addition, the anti-inflammatory effect of liraglutide has been shown in the heart of diabetic rats and in the brain of rats with Alzheimer's disease (Noyan-Ashraf et al., 2013; Parthsarathy & Hölscher, 2013).

Based on this information, the protective effect of liraglutide in testicular I/R damage is worthy of interest. There is no information about the protective effect of liraglutide on testicular I/R injury. Due to the effects of liraglutide mentioned above, this molecule will be protective in testicular ischaemia reperfusion injury in a rat model. In present study, our primary objective is to evaluate the effect of liraglutide on histopathological, immunohistochemical and biochemical changes in testis tissue of rats.

2 | MATERIAL AND METHODS

2.1 | Experimental design

The experimental study was made a total of 28 male Wistar-Albino rats, weighing between 200 and 300 gr. During the experiment, the rats were given enough water and rat feed with free access. The rats were kept in cages in groups a controlled room with 20–22°C constant temperature and twelve-hour light–dark cycle in groups before the experiment. Ethics committee approval was obtained by applying to the Istanbul University Animal Experiments Local Ethics Committee before the experiment (02/08/2017-157472). Rats were separated into four groups randomly, each group involving of seven rats.

2.2 | Surgical procedure

After anaesthesia was applied by using xylazine/ketamine (10/50 mg/kg, i.p.) the left testicle and spermatic cord were released by making a midscrotal incision. The orchiectomy procedure was performed by clamping the left testicle. The testis was numbered and placed in the Bouin solution for histopathological study. The required amount of testis was removed from the orchiectomy material with the help of scalpel and enumerated for biochemical examination. Then, the rats were killed by taking blood from the heart under anaesthesia.

Group A was the control group. Rats underwent simple scrotal orchiectomy and then killed.

Group B was the sham operation group. In this group, without creation of torsion model, the testis was placed in scrotum after dissecting the testicle according to anatomical position and scrotum was repaired with 6/0 ''polypropylene suture (Prolene™ 6-0, Ethicon®). After 120 min, orchiectomy was performed under anaesthesia. In order to investigate whether the effects of the surgical stress we created affected the outcome of our experiment, the control and sham groups were created and examined separately.

Group C was the I/R model without drug administration. Experimental acute testicular torsion model was formed by rotating left testis cord elements 720 degrees clockwise and with fixed testis on the inner surface of scrotum with 6/0 polypropylene suture. After 120 min of torsion, the scrotum was opened from the incision line and the testis was detorsed and reperfusion was achieved. The scrotum was closed again with 6/0 polypropylene suture. After waiting for 4 hours of reperfusion, orchiectomy was performed.

Group D was the I/R model and injected the normal daily use dosage 0.6 mg/kg liraglutide IP. An experimental acute testicular torsion was formed like with group C. Additionally, 0.6 mg/kg liraglutide was applied IP thirty minutes before detorsion in this group.

2.3 | Biochemical procedures

At the end of the study, samples were taken to −80°C and stored until the time of biochemical analysis.

Malondialdehyde (MDA) was mentioned to as thiobarbituric acid reagent and evaluated on the spectrophotometer at 532 nm with thiobarbituric acid (Wasowicz, Neve, & Peretz, 1993). Nitric Oxide (NO) levels in tissue were measured according to the method of Navarro-Gonzálvez et al. (1998). Superoxide dismutase (SOD) activity is calculated by degradation of nitrobluete trazolium (NBT) of superoxide produced by the xanthine/xanthine oxidase system (Sun, Oberley, & Li, 1988). Glutathione peroxidase (GPx) reduces the amount of cumene hydroperoxide during the oxidation of glutathione (GSH) to glutathione disulfide (GSSG). GSSG is produced by the consumption of NADPH, and the reduce in the amount of NAPDH is proportional to GPx activity. After studying the kit procedure, kinetic measurement was taken by spectrophotometer at 340 nm for 5 min (Hasegawa et al., 2002). Catalase (CAT), via catalytic activity, decomposes H_2O_2 into water and oxygen. The CAT enzyme activities in the tissues were determined according to the method described by Aebi (1984).

2.4 | Histopathological evaluation

All histopathological evaluations were done double-blinded. Testicular tissue specimens were evaluated in Bouin solution, subjected to routine tissue follow-up, embedded in paraffin blocks. After that, 5 μm thick sections of paraffin-embedded tissue blocks

were viewed in rotary microtome (Leica RM2255) for histopathological examination. It was then stained with Haematoxylin & Eosin (H&E) stain and analysed under a light microscope (Olympus BX50). For histopathological evaluations, the Johnsen scoring system, which determines spermatogenesis activity in testis, was used. Using a 10X objective lens, at least 50 tubules were examined for each group. Every seminiferous tubule profiles were given a score from one to ten on the base of the criteria by Johnsen (Johnsen, 1970).

2.5 | Immunohistochemical staining

Testis sections previously immunised for immunohistochemical labelling with primary antibodies to inducible nitric oxide synthetase (iNOS) and apoptosis protease activating factor 1 (Apaf-1) were stained in accordance with the manufacturer's instructions. Immunohistochemical staining was examined by a light microscope. Immunological reaction was scored in accordance with the intensity and degree of staining in the study.

2.6 | Statistical analysis

IBM SPSS Statistics 22 for statistical analysis (SPSS IBM) programmes were used to evaluate the finding in this study. When evaluating the study data, the suitability of the parameters to normal distribution was examined with the Shapiro–Wilks test. For comparison of quantitative data, the Kruskal–Wallis test and Mann–Whitney U test were used. Significance was evaluated at $p < .05$.

3 | RESULTS

No noticeable signs of external toxicity or deaths were observed in the groups of rats. The biochemical, histopathological and immunohistochemical results were quite analogous for the control and sham groups, and we decided to consider them without distinction and report only the control group.

3.1 | Biochemical results

The CAT, SOD, GPx, NO and MDA values of testicular tissue samples are shown in Table 1. Accordingly, it was observed that CAT and SOD values decreased in group C, but this decrease was not statistically significant ($p > .05$). In addition, CAT values were increased in group D compared to group C, but this increase was not statistically significant ($p > .05$). NO and MDA values increased significantly in group C ($p < .05$). In addition, the values decreased significantly in the group D compared with group C ($p < .05$). GPx values decreased significantly in group C ($p < .05$). In addition, GPx values were significantly increased in group D ($p < .05$).

3.2 | Histopathological results

In the histopathological examination of the testicular tissues of the healthy control group, testicular tubules were found to be regular and spermatogenesis was normal (Figure 1A). In the tissue samples of the sham group, mild hyperaemia, oedema and vacuolar degeneration were detected (Figure 1B, Table 2, Table 3). The inflammatory markers were listed as double-blind between 0 and 3 according to their findings by two separate pathologists.

According to histopathological examination, acute inflammation findings such as oedema bleeding were found to be highest in the I/R group compared with the other groups. In addition to vascular response, necrosis, vacuolar degeneration (Figure 1D) and spermatic giant cell formation (Figure 1C) were significantly increased in the study (Table 2, Table 3).The same parameters were determined between groups C and D. Inflammation findings in group C were higher than group D. (Figure 1E,F).

When the Spermatogenesis Johnsen score was evaluated double-blinded, it was observed that the mean values were lower in the group C. Spermatogenesis scores were also low in the group D. In the control and sham groups, the Johnsen score evaluation showed that spermatogenesis activities were not affected (Figure 2).

It was observed that Johnsen scores decreased significantly in group C compared to the sham and control groups ($p < .05$). In

TABLE 1 Comparison of biochemical parameters in tissue between groups

parameters	Control	Sham	I/R	I/R+liraglutide
CAT (nmol/gr)	36.6±3.7	32±4.5	31±1.9	33.1±3.9[a]
GPx (nmol/gr)	7.6±2.8	6.6±3.3	1.7±1.2	7.8±9.9[b]
MDA (nmol/gr)	41.7±19.4	57.4±28	113.7±44.6	46.4±26.9[c]
SOD (U/gr)	1.7±0.6	1.3±0.6	0.9±0.7	1.1±0.7[a]
NO(nmol/gr)	18±8	18.5±9.9	37.4±7.1	21.7±10.6[c]

Notes: Values are expressed as mean±SD for seven rats in each group.

[a]CAT values were increased in group D compared with group C, but this increase was not statistically significant ($p > .05$).

[b]GPx values were significantly increased in group D compared with group C ($p < .05$).

[c]NO and MDA values decreased significantly in group D compared with group C ($p < .05$).

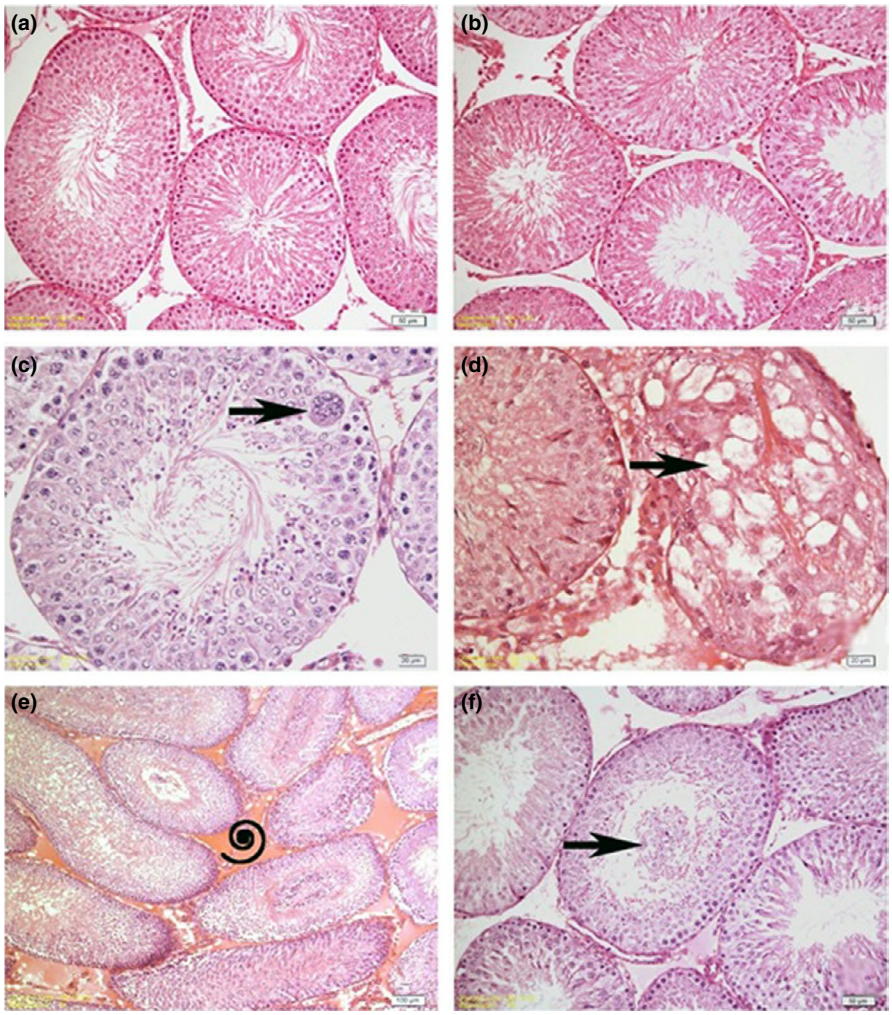

FIGURE 1 Microscopic findings of testis tissue of experimental groups. (A) Testis tissue section of healthy control group, Bar = 50 μm, H&E. (B) Testicular tissue of Sham group, Bar = 50 μm, H&E. (C) Testicular tissue of ischaemia-reperfusion group, presence of spermatic giant cell (arrow), Bar = 20 μm, H&E. (D) Testicular tissue of ischaemia-reperfusion group, severe tubular degeneration (arrow), Bar = 20 μm, H&E. (E) Ischaemia-reperfusion + liraglutide testicular tissue, tubular degeneration, intense proteinous oedema in the intertubular area (spiral), Bar = 100 μm, H&E. (F) Ischaemia-reperfusion + liragglutide testicular tissue, tubular degeneration, luminal desquamation (arrow), Bar = 50 μm, H&E

TABLE 2 Light microscopy findings of inflammatory evaluations examined histopathologically between groups

	OEdema					Bleeding			
	n	none	+	++	+++	none	+	++	+++
Control	7	6	1	0	0	7	0	0	0
Sham	7	4	2	1	0	6	1	0	0
I/R	7	0	0	0	7	0	0	0	7
I/R+Liraglutide	7	1	3	3	0	4	1	1	1

n: total number of rats each group, +: mild oedema or bleeding reported, ++: moderate oedema or bleeding reported, +++: severe oedema or bleeding reported

Acute inflammation findings such as oedema bleeding were found to be highest in the ischaemia-reperfusion group compared to the other groups

addition, Johnsen scores in group D increased significantly compared with group C ($p < .05$) (Figure 2).

In immunohistochemical staining, while Apaf-1 positivity was mild and moderate in the control (Figure 3A) and sham (Figure 3B) groups, Apaf-1 expression was significantly increased in the experimental groups compared to the healthy control group. Especially, Apaf-1 expression positivity was determined at a +3 level in all individuals of ischaemia-reperfusion group (Figure 3C). In the liraglutide treatment group, the mean positivity was +2 (Figure 3D, Table 4).

In the evaluation of staining in terms of the iNOS expression, various levels of positivity were determined in all study groups. The iNOS expression was mildly observed in healthy subjects, but increased in all testicular tissues with ischaemic damage. The iNOS expression was found to be slightly positive in the control group (Figure 4A), whereas it was higher in the sham group than in the control group (Figure 4B). In the ischaemia-reperfusion group, iNOS positivity was found to be +3 in all subjects (Figure 4C), whereas in the liraglutide treatment group, iNOS expression

TABLE 3 Light microscopy findings of inflammatory evaluations examined histopathologically between groups

	Giant cell					Vacuolar degeneration			
	n	none	+	++	+++	none	+	++	+++
Control	7	7	0	0	0	6	1	0	0
Sham	7	7	0	0	0	6	1	0	0
I/R	7	4	2	1	0	5	1	1	0
I/R+Liraglutide	7	6	1	0	0	5	2	0	0

n: total number of rats each group, none: pathologists reported 0, +: pathologists reported 1, ++: pathologists reported 2, +++: pathologists reported 3 giant cell or vacuolar degeneration.

Giant cell and Vacuolar degeneration were found to be highest in the ischaemia-reperfusion group compared to the other groups

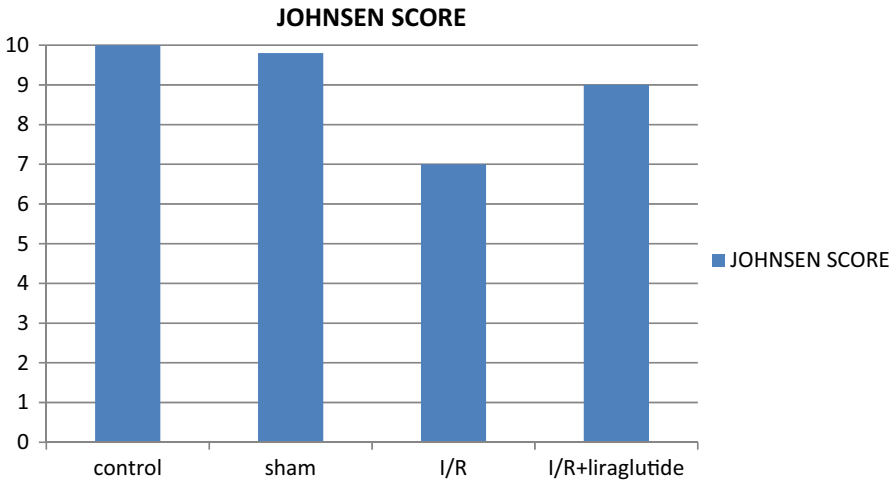

FIGURE 2 Comparison of Johnsen scores between groups. Johnsen scores decreased significantly in group C compared with sham and control groups. In addition, Johnsen scores in group D increased compared with group C

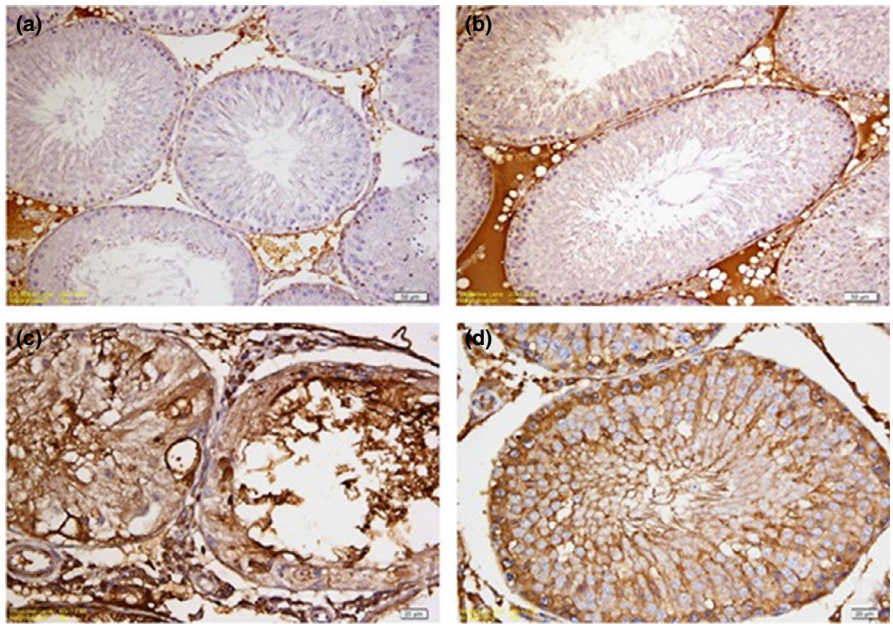

FIGURE 3 Apaf-1 staining levels between groups. (A) Testis tissue of healthy control group, Apaf-1 mild positive staining, Bar = 50 μm. (B) Testicular tissue of Sham group, Apaf-1 moderate positive staining, Bar = 50 μm. (C) Testis tissue belonging to ischaemia-reperfusion group, Apaf-1 strong staining, Bar = 20 μm. (D) Testicular tissue belonging to ischaemia-reperfusion + liraglutide group, Apaf-1 strong staining, Bar = 20 μm

was relatively lower than in the ischaemia-reperfusion group. (Table 4).

4 | DISCUSSION

Testicular torsion is an urgent urological condition that occurs especially in young males and may affect testicular function and reproductive health. Ischaemia and reperfusion caused by detorsion of the torsion testis lead to biochemical and morphological changes in the tissue. Free oxygen radicals resulting from reperfusion are held responsible for reperfusion damage (Moghimian et al., 2016). If surgical treatment does within six hours of the presenting pain, there is a decent chance of saving the affected testicle, as 90%-100% testicles will be saved. If surgical treatment does within 6–12 hr, 20%–50% testicles will be saved and if surgical treatment does within

TABLE 4 Comparison of Apaf-1 and iNOS values between groups

	Apaf-1					iNOS			
	n	none	+	++	+++	none	+	++	+++
Control	7	6	1	0	0	5	2	0	0
Sham	7	5	2	0	0	5	2	0	0
I/R	7	0	0	1	6	0	0	2	5
I/R+liraglutide	7	0	5	2	0	0	5	2	0

n: total number of rats each group, +: mild positivity, ++: moderate posivitiy, +++: severe posivity
iNOS expression was relatively lower than in the ischaemia-reperfusion group. Apaf-1 expression was significantly increased compared to the healthy control group. Apaf-1 expression positivity was determined at a +3 level in all individuals of ischaemia-reperfusion group

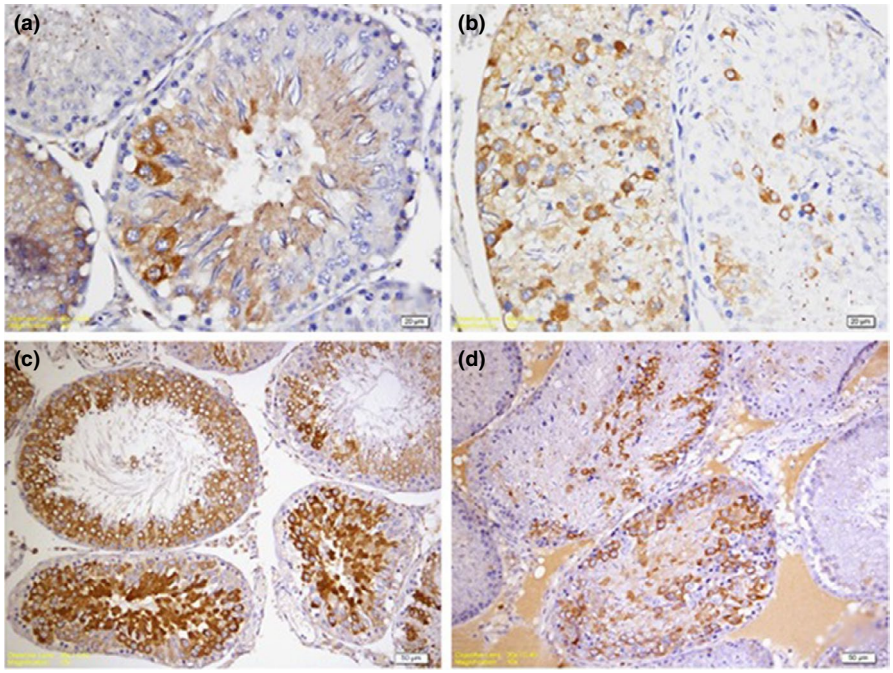

FIGURE 4 iNOS staining levels between groups. (A) Control group testis tissue, iNOS, mild positive staining, Bar = 20 μm. (B) Testicular tissue of sham group, iNOS mild positive staining, Bar = 20 μm. (C) Testicular tissue of ischaemia-reperfusion group, iNOS strong staining, Bar = 50 μm. (D) Testicular tissue belonging to ischaemia-reperfusion + liraglutide group, iNOS moderate positive staining, Bar = 50 μm

12–24 hr 0%–10% testicles will be saved (Shimizu et al., 2016). For this reason, it requires rapid surgery to minimalise germinal cell injury induced subfertility or infertility.

Rapid diagnosis and effective surgical treatment are very important to avoid ischaemic damage to the testicles. Surgical treatment is required to detorsion the testicle and redintegrate blood flow. Nevertheless, testicular reperfusion injury can also be produced by surgeon after surgical management. Although the first pathology is tissue ischaemia, I/R injury subsequent to testicular torsion–detorsion contributes to the damage. During the reperfusion phase, impairment in membrane permeability in mitochondria and cell membranes with lipid peroxidation damages enzymes, proteins and therefore DNA. With this, testicular germ cell injury caused by the ischaemia increases further (Mogilner et al., 2006).

Varied therapeutic treatment options have been discussed in the literature. Reduce in the amount of endogenous antioxidants indicated antioxidant apposition prior reperfusion might provide preservation in I/R damage on testis (Shimizu et al., 2016). To achieve this, trimetazidine, N-acetylcysteine, tadalafil and darbepoetin were used in experimental studies (Payabvash et al., 2007; Pekcetin et al., 2007; Yildirim et al., 2018). Some of these ingredients could not begin to be involved in everyday clinical use due to the lack of efficacy, the uncertainty of side effects, and the lack of adequate information about the safety and dosage of use.

Cells are protected by SOD against the destructive effects of superoxide radicals physiologically. A decrease in SOD activity after reperfusion was observed in most experimental testicular torsion studies (McCord & Edeas, 2005; Payabvash et al., 2007). In our study, in the group C, it was observed a decrease in SOD values, but this decrease was not statistically significant. In addition, in the group D, it was observed an increase in SOD values compared to group C, but this increase was not statistically significant. CAT is an enzyme created by SOD that converts hydrogen peroxide into water and oxygen in peroxisomes. CAT levels have been observed to decrease following experimental I/R study (Noske et al., 1998). In present study, in the group C, it was observed a decrease in CAT values, but this decrease was not statistically significant. In addition, in the group D, it was observed an increase in CAT values compared to group C, but this increase was not statistically significant. In the existence of hydrogen peroxide, the oxidation of the reduced

glutathione is catalysed by GPx to oxidised glutathione. GPx also reduces hydrogen peroxide created by SOD to water and oxygen in the mitochondria and cytoplasm (Jaeschke, 1995). GPx values were statistically significantly decreased in group C in our study. In addition, in group D, it was observed that the GPx values increased compared to the I/R group and this was a statistically significant increase. The latest product of lipid peroxidation is MDA, that is noted as a dependable indicator of lipid peroxidation (Cao et al., 2011). Azizollahi et al. (2011) reported increased serum MDA levels in the testicular experimental ischaemia reperfusion model in the testicular torsion group compared to the control group. In present study, MDA values increased compared to the control group and, this increase was statistically significant. Besides, it was investigated that MDA values decreased with the administration of liraglutide and, this decrease was also statistically significant. NO is a major marker of cellular demolition by virtue of necrosis or apoptosis. Ischaemia in the vascular endothelium causes an increase in NOS activity and, following this increase, contributes to the formation of superoxide radicals with the occurrence of reperfusion (Shiraishi, Naito, & Yoshida, 2001). In our study, tissue NO values in the testicles remaining ischaemic increased significantly compared to the Sham group. It was observed a statistically significant decrease in NO values by enjected liraglutide. The effect of liraglutide on biochemical values was parallel to antioxidant substances in the literature in present study.

iNOS can be produced and detected by inducing secondary messengers produced in the body in environments where ischaemia occurs in the inflammatory infection process (Chamberlain, Mansfield, & Cerra, 2008). In our study, tissue iNOS values increased statistically significant after reperfusion compared to the Sham group. At the same time, it was observed a statistically significant decrease in the iNOS values by enjected liraglutide. Apaf-1 plays a main role in mitochondrial apoptosis (Erol et al., 2009). In our study, there was a significant increase in tissue Apaf-1 values compared with the Sham group. It was observed a statistically significant decrease in Apaf-1 values by enjected liraglutide. This result supported the efficacy of liraglutide immunohistochemically.

One of the important parameters affected in testicular torsion is spermatogenesis. It has been indicated that spermatogenic function is notably damaged with respect to the Johnsen criteria in periods ranging from two hours to three months after testicular reperfusion (Ergur et al., 2008; Pekcetin et al., 2007). In present study, the Johnsen score was lowest in the I/R group.

Liraglutide is a molecule whose protective effect is proven in ischaemia reperfusion studies in organs such as heart and liver (Abdelsameea, Abbas, & Abdel Raouf, 2017; Cheng et al., 2015). In addition, the effect of reducing oxidative stress in endothelial and brain nerve cells has been shown (Batchuluun et al., 2014; Shiraki et al., 2012). It is the first study which shows the protective effect of liraglutide on testicular I/R injury in rats. The results obtained were in line with the literature knowledge in most of the findings.

Experimental animal studies are very important in establishing a new treatment modality as they provide the basis for clinical studies. In order to administer a drug to a person in a pathological situation, several stages are required. Experimental animal studies are one of these stages. In our study, limited number of data were examined, so it would not be correct to obtain a definite result from a single study, however, when the data obtained from similar experimental studies are evaluated together, a basis for clinical studies can be formed.

The study has some limitations like no evidence of long-term effects, the small sample, and the amount of biochemical and histopathological data examined is limited. Besides evaluated morphological semi-quantitative results, quantitative methods were not performed in present study.

To sum up, the protective effect of liraglutide in testicular ischaemia reperfusion injury was showed in present study. The study does not clearly demonstrate that liraglutide has perfect protective effect since significance was not demonstrated, as some of the calculations are not statistically significant. However, it is clear that even if it is not statistically significant in some calculations, it shows a close interference to the condition. This study has shown that it may be protective but it needs further research in larger animal groups. Moreover, varied experimental studies at different doses over a longer period are required.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

Abdelsameea, A. A., Abbas, N. A. T., & Abdel Raouf, S. M. (2017). Liraglutide attenuates partial warm ischemia-reperfusion injury in rat livers. *Naunyn-Schmiedeberg's Archives of Pharmacology*, *390*(3), 311–319. https://doi.org/10.1007/s00210-016-1330-7.

Aebi, H. (1984). Catalase in vitro assay methods. *Methods in Enzymology*, *105*, 121–126.

Anim, J. T., Kehinde, E. O., Prasad, A., & Varghese, R. (2005). Morphological responses of the rabbit testis to Ischemic/Reperfusion injury due to torsion. *Urologia Internationalis*, *75*(3), 258–263. https://doi.org/10.1159/000087805.

Azizollahi, S., Babaei, H., Derakhshanfar, A., & Oloumi, M. M. (2011). Effects of co-administration of dopamine and vitamin C on ischaemia-reperfusion injury after experimental testicular torsion-detorsion in rats. *Andrologia*, *43*(2), 100–105. https://doi.org/10.1111/j.1439-0272.2009.01028.x.

Batchuluun, B., Inoguchi, T., Sonoda, N., Sasaki, S., Inoue, T., Fujimura, Y., … Takayanagi, R. (2014). Metformin and liraglutide ameliorate high glucose-induced oxidative stress via inhibition of PKC-NAD(P)H oxidase pathway in human aortic endothelial cells. *Atherosclerosis*, *232*(1), 156–164. https://doi.org/10.1016/j.atherosclerosis.2013.10.025.

Cao, D., Li, H., Yi, J., Zhang, J., Che, H., Cao, J., Zhu, C. & Jiang, W. (2011). Antioxidant properties of the mung bean flavonoids on alleviating heat stress. *PLoS ONE*, *6*(6), https://doi.org/10.1371/journal.pone.0021071.

Chamberlain, C. G., Mansfield, K. J., & Cerra, A. (2008). Nitric oxide, a survival factor for lens epithelial cells. *Molecular Vision*, *14*, 983–991.

Cheng, X., Wang, X., & Teng, X. (2015). Liraglutide attenuates myocardial ischemia/reperfusion injury possibly through reducing

cardiomyocytes apoptosis and oxidation in rats. *Zhonghua Xin Xue Guan Bing Za Zhi*, *43*(3), 259–263.

Ergur, B. U., Kiray, M., Pekcetin, C., Bagriyanik, H. A., & Erbil, G. (2008). Protective effect of erythropoietin pretreatment in testicular ischemia-reperfusion injury in rats. *Journal of Pediatric Surgery*, *43*(4), 722–728. https://doi.org/10.1016/j.jpedsurg.2007.11.028.

Erol, B., Bozlu, M., Hanci, V., Tokgoz, H., Bektas, S., & Mungan, G. (2009). N79 Coenzyme Q10 treatment reduces lipid peroxidation, inducible and endothelial nitric oxide synthases and germ cell-specific apoptosis in testicular ischemia/reperfusion injury. *European Urology Supplements*, *8*(8), 596. https://doi.org/10.1016/s1569-9056(09)74853-5.

Filho, D. W., Torres, M. A., Bordin, A. L. B., Crezcynski-Pasa, T. B., & Boveris, A. (2004). Spermatic cord torsion, reactive oxygen and nitrogen species and ischemia-reperfusion injury. *Molecular Aspects of Medicine*, https://doi.org/10.1016/j.mam.2004.02.020.

Hasegawa, Y., Takano, T., Miyauchi, A., Matsuzuka, F., Yoshida, H., Kuma, K., & Amino, N. (2002). Decreased expression of glutathione peroxidase mRNA in thyroid anaplastic carcinoma. *Cancer Letters*, *182*(1), 69–74. https://doi.org/10.1016/S0304-3835(02)00069-1.

Jaeschke, H. (1995). Mechanisms of oxidant stress-induced acute tissue injury. *Proceedings of the Society for Experimental Biology and Medicine*, *209*(2), 104–111. https://doi.org/10.3181/00379727-209-43885b.

Johnsen, S. G. (1970). Testicular biopsy score count – a method for registration of spermatogenesis in human testes: Normal values and results in 335 hypogonadal males. *Hormone Research in Paediatrics*, *1*(1), 2–25. https://doi.org/10.1159/000178170.

McCord, J. M., & Edeas, M. A. (2005). SOD, oxidative stress and human pathologies: A brief history and a future vision. *Biomedicine and Pharmacotherapy*, https://doi.org/10.1016/j.biopha.2005.03.005.

Moghimian, M., Soltani, M., Abtahi, H., Adabi, J., & Jajarmy, N. (2016). Protective effect of tunica albuginea incision with tunica vaginalis flap coverage on tissue damage and oxidative stress following testicular torsion: Role of duration of ischemia. *Journal of Pediatric Urology*, *12*(6), 390.e1–390.e6. https://doi.org/10.1016/j.jpurol.2016.06.002.

Mogilner, J. G., Lurie, M., Coran, A. G., Nativ, O., Shiloni, E., & Sukhotnik, I. (2006). Effect of diclofenac on germ cell apoptosis following testicular ischemia-reperfusion injury in a rat. *Pediatric Surgery International*, *22*(1), 99–105. https://doi.org/10.1007/s00383-005-1580-9.

Navarro-Gonzálvez, J. A., García-Benayas, C., & Arenas, J. (1998). Semiautomated measurement of nitrate in biological fluids. *Clinical Chemistry*, *44*(3), 679–681. https://doi.org/10.1093/clinchem/44.3.679.

Noske, H. D., Kraus, S. W., Altinkilic, B. M., & Weidner, W. (1998). Historical milestones regarding torsion of the scrotal organs. *Journal of Urology*, *159*(1), 13–16. https://doi.org/10.1016/S0022-5347(01)63997-1.

Noyan-Ashraf, M. H., Shikatani, E. A., Schuiki, I., Mukovozov, I., Wu, J., Li, R. K., Volchuk, A., Robinson, L. A., Billia, F., Drucker, D. J., & Husain, M. (2013). A glucagon-like peptide-1 analog reverses the molecular pathology and cardiac dysfunction of a mouse model of obesity. *Circulation*, *127*(1), 74–85. https://doi.org/10.1161/CIRCULATIONAHA.112.091215.

Okada, K., Kotani, K., Yagyu, H., Ando, A., Osuga, J., & Ishibashi, S. (2014). Effects of treatment with liraglutide on oxidative stress and cardiac natriuretic peptide levels in patients with type 2 diabetes mellitus. *Endocrine*, *47*(3), 962–964. https://doi.org/10.1007/s12020-014-0246-6.

Parthsarathy, V., & Hölscher, C. (2013). The type 2 diabetes drug liraglutide reduces chronic inflammation induced by irradiation in the mouse brain. *European Journal of Pharmacology*, *700*(1–3), 42–50. https://doi.org/10.1016/j.ejphar.2012.12.012.

Payabvash, S., Salmasi, A. H., Kiumehr, S., Tavangar, S. M., Nourbakhsh, B., Faghihi, S. H., & Dehpour, A. R. (2007). Salutary effects of N-acetylcysteine on apoptotic damage in a rat model of testicular torsion. *Urologia Internationalis*, *79*(3), 248–254. https://doi.org/10.1159/000107958.

Pekcetin, C., Ergur, B. U., Kiray, M., Bagriyanik, A., Tugyan, K., Erbil, G., & Ozogul, C. (2007). The protective effects of trimetazidine on testicular ischemia and reperfusion injury in rats. *Pediatric Surgery International*, *23*(11), 1113–1118. https://doi.org/10.1007/s00383-007-2002-y.

Shimizu, S., Tsounapi, P., Dimitriadis, F., Higashi, Y., Shimizu, T., & Saito, M. (2016). Testicular torsion-detorsion and potential therapeutic treatments: A possible role for ischemic postconditioning. *International Journal of Urology*, *23*(6), 454–463. https://doi.org/10.1111/iju.13110.

Shiraishi, K., Naito, K., & Yoshida, K. I. (2001). Nitric oxide promotes germ cell necrosis in the delayed phase after experimental testicular torsion of rat. *Biology of Reproduction*, *65*(2), 514–521. https://doi.org/10.1095/biolreprod65.2.514.

Shiraki, A., Oyama, J, Komoda, H., Asaka, M., Komatsu, A., Sakuma, M., Kodama, K., Sakamoto, Y., Kotooka, N., Hirase, T., & Node, K. (2012). The glucagon-like peptide 1 analog liraglutide reduces TNF-α-induced oxidative stress and inflammation in endothelial cells. *Atherosclerosis*, *221*(2), 375–382. https://doi.org/10.1016/j.atherosclerosis.2011.12.039.

Sun, Y., Oberley, L. W., & Li, Y. (1988). A simple method for clinical assay of superoxide dismutase. *Clinical Chemistry*, *34*(3), 497–500. https://doi.org/10.1093/clinchem/34.3.497.

Wasowicz, W., Neve, J., & Peretz, A. (1993). Optimized steps in fluorometric determination of thiobarbituric acid- reactive substances in serum: Importance of extraction pH and influence of sample preservation and storage. *Clinical Chemistry*, *39*(12), 2522–2526. https://doi.org/10.1093/clinchem/39.12.2522.

Yildirim, C., Yuksel, O. H., Urkmez, A., Sahin, A., Somay, A., & Verit, A. (2018). Protective effects of Tadalafil and darbepoetin against ischemia - reperfusion injury in a rat testicular torsion model. *International Braz J Urol*, *44*(5), 1005–1013. https://doi.org/10.1590/S1677-5538.IBJU.2018.0200.

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