

# The long-term protective effect of tadalafil on spermatogenesis following testicular ischemia-reperfusion injury in a rat model

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## Research article

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# Abstract

## Background

Testicular torsion is a urological emergency in which misdiagnosis and inappropriate treatment can lead to testicular atrophy and male infertility owing to ischemia-reperfusion injury (IRI). Although experimental studies of testicular torsion have been preceded, promising therapeutic agents based on the long-term effect for spermatogenesis have not been identified in testicular ischemia reperfusion injury (IRI) animal model. Tadalafil, one of the phosphodiesterase-5 inhibitors commonly used in the treatment of erectile dysfunction, has recently reported a protective effect against IRI in several organs. In this study, we evaluated the long-term protective effect of tadalafil for spermatogenesis in a rat testicular IRI model.

## Methods

Forty-eight adolescent Sprague–Dawley rats were divided into 6 groups (A-F). Sham operation was performed in group A. Group B received surgical 720-degree torsion of the left testis without any medication. Groups C, D, E, and F were operated surgical torsion with tadalafil at varying doses (0.3 mg/kg, 1.0 mg/kg) and durations (single or daily administration for 4 weeks). Detorsion was performed after 3 hour of torsion in all rats except the sham group. Four weeks after operation, both testes were evaluated of spermatogenesis using Johnsen scoring. To evaluate the protective effect of tadalafil against oxidative stress by IRI, malondialdehyde (MDA) and superoxide dismutase (SOD) level were analyzed via ELISA in both testes 4 hour after detorsion in the same experiments as in group A, B, and C.

## Results

For the evaluation of spermatogenesis according to doses, the groups with high-dose tadalafil showed a higher Johnsen scores than low-dose counterparts. The groups with daily administration for 4weeks were observed a higher Johnsen scores than those given a single administration. Furthermore, molecular markers (MDA and SOD) related with oxidative stress and histopathologic findings showed remarkable improvement after tadalafil administration.

## Conclusion

Tadalafil alleviated long-term deterioration of spermatogenesis and oxidative stress by restoring antioxidant status after testicular IRI rat model. Furthermore, it demonstrated a protective effect against testicular IRI in a time- and dose-dependent manner.

## Background

Testicular torsion is a urological emergency caused by the twisting of the spermatic cord, leading to a reduction in blood flow that results in arterial obstruction, ischemia and gonadal necrosis [1, 2]. Generally speaking, it is one of the common genital diseases among adolescent boys – estimated to afflict 1 in 4000 younger than 25 years of age [3], and therefore should be corrected promptly to prevent the development of ipsilateral testicular dysfunction and infertility later in life [4]. Surgical intervention by counter-rotating the testis is the standard method of treatment; however, this can give rise to inevitable ischemia-reperfusion injury (IRI) [5–13], which impairs spermatogenesis and triggers production of reactive oxygen species (ROS) [6, 14–17].

Salvage rates have been reported in 30–50% and impaired spermatogenesis occurs in most patients with testicular torsion despite considerable efforts [16, 17]. Unfortunately, to date, there is no established standard treatment, except surgical reduction, for testicular torsion-induced injury. Theoretically, promising therapeutic candidates to prevent complications arising from testicular torsion should ameliorate ischemic injury, promote spermatogenesis, and regulate harmful immune response. Although various therapeutic agents have shown some benefits in reducing oxidative stress as short-term results in several experimental studies, appropriate pharmacological treatments based on the benefit of long-term spermatogenesis still need to be developed.

Phosphodiesterase-5 inhibitors (PDE5is) are commonly used to treat erectile dysfunction in current urology practice [18]. The inhibition of phosphodiesterase enzymes increases cyclic guanosine monophosphate (cGMP) levels in the tissue, resulting in relaxation of smooth muscles. Loosened the smooth muscles of vessel by PDE5is increases the local perfusion and dilation of the vessels and inhibits platelet aggregation, and also some studies have suggested that PDE5is might have positive effect in the prevention of IRI [19, 20]. Recently, experimental studies have suggested that PDE5i has a protective effect against IRI by regulating the antioxidant activity of several organs and disease models [6, 20–22]. Furthermore, several other studies have investigated the protective effect of testicular ischemia–reperfusion injury in a rat testicular torsion model using different PDE5i agents, such as sildenafil, tadalafil, and vardenafil [23–29]. Of PDE5is, Tadalafil is frequently used as a long-term maintenance therapy with once daily administration to maintain a steady-state blood concentration compared with other PDE5is. Nevertheless, there were few studies regarding the long-term protective effect of tadalafil for spermatogenesis in testicular IRI model.

In this study, we evaluated the protective effect of tadalafil against testicular damage for long-term spermatogenesis in a rat testicular torsion-induced IRI model.

## Methods

This study was approved by the institutional animal care and use committee of College of Medicine in Yeungnam University (YUMC-AEC2018-029). All surgeries were performed under anesthesia, and all possible efforts were made to minimize suffering.

In total, 72 male healthy 6- to 8-week-old Sprague–Dawley rats (200–250 g) correlating with adolescent period in humans were purchased from KOATECH Co. (Pyeongtaek-si, Gyeonggi-do, Republic of Korea). According to 3R (reduction, replacement, refinement) of Institutional Animal Care and Use Committee (IACUC), we chose the minimal number of animals used. A 1-week acclimatization period was allowed before any surgical procedure. All rats had free access to food and water under a 12-hour light/dark cycle. All rats were anesthetized via intramuscular injection of Rumpun and Zoletil once with the addition of one-third of the initial dose if anesthesia was not attained. We euthanized rats using CO<sub>2</sub> gas or cervical dislocation. In a previous study, we identified the aggravated testicular damage in ischemic time dependent manner and the serious deterioration of spermatogenic activity developed 3 hour after ischemia in a rat testicular torsion model [31]. Therefore, we selected 3 hour of ischemia as the torsion duration in this study. Rats received surgical torsion for 3 hour and subsequent detorsion of left testis. After left scrotal incision, unilateral testicular torsion was made by a 720-degree clockwise rotation of the left testis followed by the fixation to the scrotal wall with 4 - 0 silk suture (Fig. 1). After maintaining the torsion state for 3 hour, the testis was counter-rotated to its natural position and reinserted in the scrotum.

To evaluate the long-term effects of tadalafil on spermatogenesis, 48 healthy rats were randomly allocated into six groups (A-F) as follows:

Group A sham operation (surgical incision without testicular torsion/detorsion)

Group B 3 hour torsion/detorsion

Group C 3 hour torsion/detorsion with low-dose tadalafil single intraperitoneal injection (single intraperitoneal injection of 0.3 mg/kg tadalafil dissolved in 0.9% NaCl 1 hour before detorsion)

Group D 3 hour torsion/detorsion with low-dose tadalafil once daily administration for 4weeks (single intraperitoneal injection 1 hour before detorsion + daily medication orally of 0.3 mg/kg tadalafil dissolved in 0.9% NaCl)

Group E 3 hour torsion/detorsion with high-dose tadalafil single intraperitoneal injection (single intraperitoneal injection of 1.0 mg/kg tadalafil dissolved in 0.9% NaCl 1 hour before detorsion)

Group F 3 hour torsion/detorsion with high-dose tadalafil once daily administration for 4weeks (single intraperitoneal injection 1 hour before detorsion + daily medication orally of 1.0 mg/kg tadalafil dissolved in 0.9% NaCl).

In all rats, bilateral orchiectomies and testicular tissue sampling were performed 4 weeks after 3 hour torsion-detorsion procedures to evaluate spermatogenesis.

And we also evenly randomized 24 healthy rats into three groups (G-I) to evaluate the protective effect of tadalafil against oxidative stress.

Group G sham operation (surgical incision without testicular torsion/detorsion)

Group H 3 hour torsion/detorsion

Group I 3 hour torsion/detorsion with low-dose tadalafil single intraperitoneal injection (single intraperitoneal injection of 0.3 mg/kg tadalafil dissolved in 0.9% NaCl 1 hour before detorsion)

In all rats of three group (G-I), bilateral orchiectomies and testicular tissue samplings for histopathological/molecular analysis were performed 4 hour after 3 hour torsion-detorsion procedures.

Excised testes were paraffin embedded and stained with hematoxylin and eosin. Histopathological analysis was evaluated independently by a single pathologist blinded to the rat experimental group using a light microscope. The Johnsen scoring system was used to evaluate long-term spermatogenesis between groups A through F [32]. The value of Johnsen score in each testis was the mean point value from at least 10 seminiferous tubules. The severity of germ cell injury was qualified via Johnsen score from 10 to 1 points, as shown in Table 1.

Table 1  
Johnsen Scoring System for Evaluating Testicular Damage

Johnsen score	Description of histological criteria
10	Full spermatogenesis
9	Slightly impaired spermatogenesis, many late spermatids, disorganized epithelium
8	Less than five spermatozoa per tubule, few late spermatids
7	No spermatozoa, no late spermatids, many early spermatids
6	No spermatozoa, no late spermatids, few early spermatids
5	No spermatozoa or spermatids, many spermatocytes
4	No spermatozoa or spermatids, few spermatocytes
3	Spermatogonia only
2	No germinal cells, Sertoli cells only
1	No seminiferous epithelium

To analyze the protective effect of tadalafil against oxidative stress between group G–I, malondialdehyde (MDA) and superoxide dismutase (SOD) level were analyzed via ELISA in both testes 4 hour after detorsion. Molecular analysis, such as MDA and SOD, was assessed in a left to right ratio.

We used the lipid peroxidation assay kit (BioVision, K739–100, USA) for MDA assessment in accordance with the appropriate procedure according to the manufacturer's instructions. In brief, testicular tissues (10 mg) were homogenized in MDA Lysis Buffer (with 3 µl BHT (100x), then centrifuged (13,000 x g, 10 min) to obtain supernatant. Each sample was added 600 µl of TBA reagent. The mixtures were incubated at 95 °C for 60 minutes. After cooling with ice, 300 µl of n-butanol was added and centrifuged

(3 min at 16,000xg). N-butanol was removed and the MDA–TBA adduct was placed into a 96-well plate and absorbance was measured at 532 nm. MDA content was calculated with MDA standards.

SOD activity was analyzed by a commercially available kit from Cayman Chemicals, USA (Cat no 706002). Briefly, approximately 100 mg of testicular tissue was homogenized in 20 mM HEPES buffer containing 1 mM EGTA, 210 mM mannitol and 70 mM sucrose. After homogenization, homogenates were centrifuged at 1500 x g for 5 min at 4 °C. The supernatant was then obtained. The supernatant was diluted with sample buffer (10 x diluted) 50 times to produce absorbances within the linear range of the standard curve. Analysis was carried out according to the manufacturer's instructions, read at 450 nm. Molecular analysis, such as MDA and SOD, was assessed in a left to right ratio.

The one-way analysis of variance (ANOVA) and Tukey's post-hoc test after one-way ANOVA were used for statistical analyses. All values were expressed as the mean  $\pm$  SD. Results are representative of at least three experiments. All data were reported as the sample mean  $\pm$  SD, and p values less than 0.05 were considered significant.

## Results

Figure 2 shows the representative gross findings of the extracted left testes and the mean value of maximal longitudinal length of the left testicle compared with that of the right between groups A through F. Group D, E, and F showed significantly higher longitudinal length ratio of left to right testis than group B (group A,  $1.013 \pm 0.043$ ; group B,  $0.313 \pm 0.063$ ; group C,  $0.372 \pm 0.050$ ; group D,  $0.385 \pm 0.040$ ; group E,  $0.472 \pm 0.094$ ; group F,  $0.510 \pm 0.010$ ). In addition, tadalafil alleviated testicular atrophy after testicular IRI depending on dose and duration. Figure 3 showed the histopathological findings and Johnsen score of groups A–F. Although the testes in the sham group (group A) showed normal morphology, those in group B showed serious testicular damage, including severe edema, apoptotic bodies, venular ectasia, and lobular coagulative necrosis. However, Groups C–F (with tadalafil administration) showed improvement in histopathological findings compared with group B. The histopathological findings among Groups C–F also showed improvement depending on the dose and duration of tadalafil. The Johnsen scores of group A (sham group), B (control group), C (low-dose tadalafil/single injection), D (low-dose tadalafil/daily administration), E (high-dose tadalafil/single injection), and F (high-dose tadalafil/daily administration) were observed mean  $10 \pm 0$ ,  $2.3 \pm 1.2$ ,  $4.1 \pm 2.9$ ,  $5.3 \pm 2.3$ ,  $4.4 \pm 1.8$ , and  $7.4 \pm 1.6$ , respectively. With regard to the dose, the groups with high-dose tadalafil showed a higher Johnsen score than those with low-dose tadalafil (group C,  $4.1 \pm 2.9$  versus group E,  $4.4 \pm 1.8$ ;  $p = 0.836$ ; group D,  $5.3 \pm 2.3$  versus group F,  $7.4 \pm 1.6$ ,  $p = 0.047$ ). In addition, regarding the duration of tadalafil administration, the groups with daily administration for 4 weeks showed a higher Johnsen score than those with a single injection (group C,  $4.1 \pm 2.9$  versus group D,  $5.3 \pm 2.3$ ;  $p = 0.396$ ; group E,  $4.4 \pm 1.8$  versus group F,  $7.4 \pm 1.6$ ,  $p = 0.003$ ). The spermatogenic activity of the right testes did not deteriorate in all rats. Furthermore, there was no meaningful difference with regards to the right testis among all groups.

Histological evaluation of testicular tissues revealed normal tubular structures with the interstitium in group G (sham operation). In group H (control group), serious degeneration, including disorganization of tubular epithelium interstitial edema, hemorrhage, and less distinct seminiferous tubule borders, was found when compared with group G. In group I, tadalafil administration mitigated the deterioration of tubular histology compared with group H. In addition, molecular markers related to oxidative stress (MDA and SOD) showed a significant difference between groups G–I. MDA levels was significantly decreased (group G,  $93.5 \pm 23.7$ , group H,  $177.3 \pm 34.5$ , group I,  $107.3 \pm 38.4$ ;  $p < 0.001$  between groups G and H and  $p = 0.002$  between groups H and I) and SOD levels was significantly increased in groups G and I compared with group H (group G,  $102.3 \pm 7.5$ , group H,  $79.2 \pm 10.0$ , group I,  $115.5 \pm 9.5$ ;  $p < 0.001$  between groups G and H and  $p < 0.001$  between groups H and I; Fig. 4). Molecular markers related to oxidative stress and histopathological findings showed remarkable improvement after tadalafil administration.

## Discussion

In this study, we investigated the protective effect of tadalafil in a rat testicular torsion-induced IRI model. We observed that tadalafil attenuated oxidative stress caused by testicular torsion and had a protective effect against testicular IRI for long-term spermatogenesis in a time- and dose-dependent manner. To our knowledge, this is the first study on the protective effects of PDE5i dose and duration on testicular IRI. As a clinical implication, our data indicate that administering tadalafil to patients with testicular torsion may be beneficial against IRI in the early- as well as long-term duration.

Twisting of the spermatic cord, called testicular torsion, is not rare emergent urological condition occurring in adolescent males. Testicular torsion requires prompt diagnosis and surgical detorsion known as the current standard management for preventing histological damage and reduction of male reproductive ability [2]. Several studies have identified possible mechanisms for the progression of this emergent disease responsible for testicular atrophy and altered spermatogenesis [5–12]. The main pathophysiological consequence of testicular torsion is ischemia and reperfusion, which are generated by the twisting of the spermatic cord followed by detorsion [13]. Reperfusion involves the generation of toxic ROS with the return of blood flow after a period of ischemia. Subfertility after unilateral testicular torsion exists in nearly 40% of cases, and semen analysis may result in abnormal results in more than half of the patients with testicular torsion during long-term follow-up [4, 33]. Unfortunately, there is no established standard treatment for testicular torsion-induced injury without surgical detorsion to date. Unfortunately, to date, there is no established standard treatment for testicular torsion-induced IRI without surgical reduction. Therefore, there is a search for new promising therapeutic approaches to attenuate related pathological signals. Although several therapeutic agents have shown some benefit in reducing testicular IRI, appropriate pharmacological treatments for clinical practice still need to be developed.

Several studies have attempted to suppress testicular damage via medications and surgical maneuvers, such as postconditioning in an animal model with torsion-induced IRI [24–29, 34–44]. However, previous studies have usually focused on the change of molecular markers and histopathological change in early phase, mostly within hour, after ischemia-reperfusion injury. Most studies that have evaluated PDE5i as

an attenuating medication have performed similar experimental methods with the torsion lasting 2–4 hour, followed by a detorsion period within 4 hour, following which the rats were sacrificed and biochemical and histopathological analysis after bilateral orchiectomy was performed. The biochemical markers of oxidative stress, such as MDA, SOD, and glutathione peroxidase, were measured owing to their effects in the early phase (several hours). Although the early phase after detorsion is an appropriate time to assess changes in substances related to oxidative stress, it is a questionable period for the assessment of spermatogenesis. Generally, testicular torsion occurs during puberty; however, it is meaningful to measure spermatogenesis in terms of fertility in adulthood. In addition, testicular torsion results in testicular epithelial tissue degeneration via apoptosis secondary to testicular ischemia and reperfusion [36]. This composite pathological cascade is responsible for testicular atrophy and impaired spermatogenesis observed at later stages. Therefore, it is desirable to operate testicular IRI in the adolescence period and subsequently assess the histopathological changes regarding spermatogenesis in adult period in animal study. Previously, we conducted a study to identify the ischemic time of when a long-term serious histopathological change occurs after testicular IRI in adolescent rats. We confirmed via histopathological analysis 4 weeks after testicular IRI that a serious deterioration in spermatogenic activity was induced after 3 hour of ischemia rather than after shorter ischemic times. Therefore, we selected 3 hour of ischemia as the duration of torsion for confirming the long-term spermatogenic results of the current study [31].

Tadalafil is one of the mostly used PDE5i to treat erectile dysfunction and maintain a steady-state blood concentration that repairs damaged endothelium and improves erectile function, while sildenafil, vardenafil, and udenafil are more rapidly absorbed. Therefore, tadalafil is frequently used once daily as a long-term maintenance therapy [18, 19]. Inhibition of PDE5 enzyme may cause increased cGMP levels, ultimately resulting in smooth muscle relaxation [18, 19]. There have been several reports regarding the protective effects of PDE5i therapy against IRI in several organs, including kidney, heart, intestine, and lung tissues, in experimental models via anti-inflammation, anti-ROS, and anti-apoptosis activities [19, 20]. Furthermore, several studies have reported that various types of PDE5is may have a protective effect on testicular torsion-induced IRI animal models [23–29, 42]. However, there have been contradictory results regarding the effect of PDE5is on testicular torsion animal models. In agreement with our results, Ozmerdiven et al [25], Ameli et al [26], Yildirim et al [27], and Wu et al [42] have reported the beneficial effects of tadalafil administration in testicular IRI rat model. They reported increased antioxidant enzyme levels, decreased lipid peroxidation levels, germ cell apoptosis, and attenuated histopathological results in the early phase. Similar results can be also found in other studies based on sildenafil and vardenafil [23, 24, 29, 45]. On the other hand, some studies have shown controversial results regarding the effects of PDE5is after testicular IRI. Utsun et al reported that sildenafil and vardenafil caused exaggerated testicular apoptosis and increased nitric oxide synthase levels after 1 hour of ischemia and 2 hour of reperfusion injury [39]. Additionally, Istanbuluoglu et al suggested that vardenafil worsened histopathological changes related to oxidative stress and had no protective effect on testicular IRI in a pig model [28]. Regarding on the conflicting outcomes of PDE5i in testicular IRI, we assumed that the differences were caused because of varying torsion and reperfusion times, doses of PDE5is, and animal

species used in the studies. In addition, it is well documented that changes in the testes depend on torsion duration; however, the exact time presenting serious testicular deterioration is unclear in previous studies. In the current study, we identified 3 hour as the exact torsion time related to serious damage via our previous study and confirmed the protective effect of tadalafil based on an established torsion-induced IRI rat model.

Oxidative stress is caused by an imbalance in the production of oxidants and the scavenger, a product of the defense system. Although ROS is maintained at physiologically low levels by the endogenous antioxidant defense system in tissue under normal conditions, overgeneration of oxidative stress, in which ROS generation exceeds the defense mechanisms capacity to control it, such as in the course of testicular ischemia and reperfusion, contributes to reversible or irreversible cell injury [46, 47]. Several studies have established that testicular torsion-induced IRI increases oxidative stress and decreases the antioxidant scavenger [23–29, 42]. MDA is a stable end product of lipid peroxidation generated by ROS and is usually used as an indirect indicator of oxidative damage [48, 49]. In addition, SOD is a key component in cell growth, differentiation, and protection and acts as one of the enzymatic antioxidant defense systems against ROS and oxidative damage [47]. The results of our study showed that tadalafil can protect spermatogenic activity in the late phase as well as attenuate oxidative stress markers in the early phase. This may be due to the antioxidant property and ability of tadalafil in testicular torsion-induced IRI model [19]. These results agree with those of previous studies using tadalafil [25–27, 42]. In addition, our study demonstrated that tadalafil can alleviate histopathological damage after testicular torsion-induced IRI in a time- and dose-dependent manner in the late phase. Although further studies are needed regarding the exact mechanism, our study supports the notion that tadalafil, which is suitable for dose escalation and long-term use, has encouraging preventive effects with a positive correlation with dose and exposure duration against testicular injury. We assumed that this phenomenon is due to the persistent and cumulative antioxidant property of tadalafil as an excellent scavenger against free radical generation.

The data on damage to the contralateral testis after ischemia–reperfusion injury are conflicting. Some studies have reported contralateral testis impairment after ipsilateral testicular ischemia–reperfusion injury in experimental models [50, 51]. Although the exact mechanism of contralateral damage is not fully understood, decreased testicular blood flow by reflex sympathetic response, ROS overproduction, and autoimmunization against the spermatogonia are hypothesized to explain this phenomenon [52]. However, there were several studies that have reported no effect of testicular torsion on contralateral testis [53, 54]. Recently, Ozgur et al [24] and Unsal et al [38] observed no pathological changes in the contralateral testis after ipsilateral testicular IRI, similar to the findings of our study. This difference is thought to be a result of diverse animal models and evaluation methods for contralateral damage to the testis and should be clarified via additional studies using standardized animal models in the future.

Our study has several limitations that need to be considered. Tadalafil has a relatively long duration of action, with a higher half-life than other PDE5is. In addition, the recommended initial dose of tadalafil is 2.5–5 mg for maintenance therapy in humans and can be increased up to 20 mg. In our study using

doses of 0.3 mg/kg and 1 mg/kg, the higher dose of tadalafil showed greater efficacy; however, this dose is not within normal clinical use limits for humans. Therefore, further studies are warranted to elucidate the optimal dose and duration of tadalafil administration to maximize efficacy without possible adverse effects. Furthermore, there is concern regarding the long-term use of PDE5is because some reports have shown that chronic administration of tadalafil to rats may result in degenerative changes in the testis and a decline in semen parameters [55]. Therefore, additional studies regarding the long-term adverse effects of tadalafil itself on the normal or pathological testes will be required. Finally, further studies are essential to clarify the mechanism of chronic use of tadalafil and to establish a protocol applicable to humans for future clinical application.

## Conclusions

To our knowledge, this is the first study of the effects of PDE5is concerning dose and duration on testicular IRI. This study demonstrates that tadalafil can alleviate histopathological changes and oxidative stress by restoring antioxidant status in testicular IRI in a rat testicular torsion model. In addition, it has a protective effect against testicular IRI in a time- and dose-dependent manner for long-term spermatogenesis. As a clinical implication, our data indicate that administering PDE5is to patients with testicular torsion may be beneficial for early- and long-term follow-up, providing a promising approach that may be modified and used in the near future.

## Abbreviations

CAPE

Caffeic acid phenethyl ester

cGMP

Cyclic guanosine monophosphate

ELISA

Enzyme-linked immunosorbent assay

IRI

Ischemia-reperfusion injury

MDA

Malondialdehyde

PDE5is

Phosphodiesterase-5 inhibitors

ROS

Reactive oxygen species

SOD

Superoxide dismutase

## Declarations

## **Ethics approval and consent to participate**

This study was approved by the institutional animal care and use committee of College of Medicine in Yeungnam University (YUMC-AEC2018-029).

## **Consent for publication**

Not applicable.

## **Availability of data and materials**

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

## **Competing interests**

The authors declare that there are no conflicts of interest.

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## **Authors' contributions**

BMK: Draft or revision article

EHL: Draft or revision article

BHY: Acquisition of data

SYC: Data analysis

GSY: Data analysis

JWC: Data analysis

YSH: Interpretation of data

BSK: Interpretation of data

JYC: Interpretation of data

PHS: Interpretation of data

TGK: Conception and design

JNL: Conception and design

All authors have read and approved the manuscript.

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## References

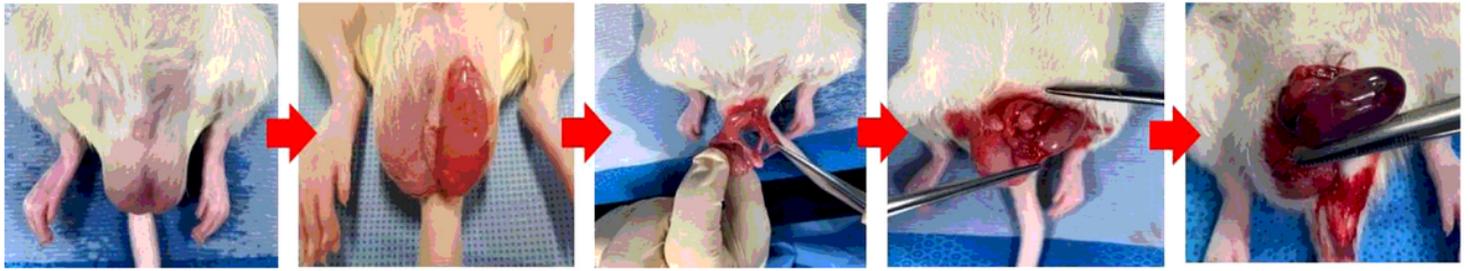
1. Jhunjhunwala JS, Desai A, Kropp KA. Torsion of the spermatic cord. An Experimental Study. *Invest Urol.* 1976;13(4):318–20.
2. Ringdahl E, Teague L. Testicular torsion. *Am Fam Physician.* 2006;74(10):1739–43.
3. Mansbach JM, Forbes P, Peters C. Testicular torsion and risk factors for orchiectomy. *Arch Pediatr Adolesc Med.* 2005;159(12):1167–71.
4. Anderson JB, Williamson RC. The fate of the human testes following unilateral torsion of the spermatic cord. *Br J Urol.* 1986;58(6):698–704.
5. Fraser LR, Duncan AE. Adenosine analogues with specificity for A2 receptors bind to mouse spermatozoa and stimulate adenylate cyclase activity in uncapacitated suspensions. *J Reprod Fertil.* 1993;98(1):187–94.
6. Filho DW, Torres MA, Bordin AL, Crezcynski-Pasa TB, Boveris A. Spermatic cord torsion, reactive oxygen and nitrogen species and ischemia-reperfusion injury. *Mol Aspects Med.* 2004;25(1–2):199–210.
7. Lysiak JJ. The role of tumor necrosis factor-alpha and interleukin-1 in the mammalian testis and their involvement in testicular torsion and autoimmune orchitis. *Reprod Biol Endocrinol.* 2004;2:9.
8. Antonuccio P, Minutoli L, Romeo C, Nicotina PA, Bitto A, Arena S, et al. Lipid peroxidation activates mitogen-activated protein kinases in testicular ischemia-reperfusion injury. *J Urol.* 2006;176(4 Pt 1):1666–72.
9. Cuervo JL, Grillo A, Vecchiarelli C, Osio C, Prudent L. Perinatal testicular torsion: a unique strategy. *J Pediatr Surg.* 2007;42(4):699–703.
10. Minutoli L, Antonuccio P, Polito F, Bitto A, Squadrito F, Di Stefano V, et al. Mitogen-activated protein kinase 3/mitogen-activated protein kinase 1 activates apoptosis during testicular ischemia-reperfusion injury in a nuclear factor-kappaB-independent manner. *Eur J Pharmacol.* 2009;604(1–3):27–35.
11. Minutoli L, Antonuccio P, Polito F, Bitto A, Squadrito F, Irrera N, et al. Peroxisome proliferator activated receptor beta/delta activation prevents extracellular regulated kinase 1/2 phosphorylation and protects the testis from ischemia and reperfusion injury. *J Urol.* 2009;181(4):1913–21.

12. Minutoli L, Antonuccio P, Squadrito F, Bitto A, Nicotina PA, Fazzari C, et al. Effects of polydeoxyribonucleotide on the histological damage and the altered spermatogenesis induced by testicular ischaemia and reperfusion in rats. *Int J Androl.* 2012;35(2):133–44.
13. Akgur FM, Kilinc K, Aktug T. Reperfusion injury after detorsion of unilateral testicular torsion. *Urol Res.* 1993;21(6):395–9.
14. Reyes JG, Farias JG, Henriquez-Olavarrieta S, Madrid E, Parraga M, Zepeda AB, et al. The hypoxic testicle: physiology and pathophysiology. *Oxid Med Cell Longev.* 2012;2012:929285.
15. Dokmeci D. Testicular torsion, oxidative stress and the role of antioxidant therapy. *Folia Med (Plovdiv).* 2006;48(3–4):16–21.
16. Visser AJ, Heyns CF. Testicular function after torsion of the spermatic cord. *BJU Int.* 2003;92(3):200–3.
17. Tryfonas G, Violaki A, Tsikopoulos G, Avtzoglou P, Zioutis J, Limas C, et al. Late postoperative results in males treated for testicular torsion during childhood. *J Pediatr Surg.* 1994;29(4):553–6.
18. van Driel MF. [Phosphodiesterase inhibitors: effectiveness and new applications]. *Ned Tijdschr Geneesk.* 2006;150(29):1613–6.
19. Kulkarni SK, Patil CS. Phosphodiesterase 5 enzyme and its inhibitors: update on pharmacological and therapeutical aspects. *Methods Find Exp Clin Pharmacol.* 2004;26(10):789–99.
20. Das A, Durrant D, Salloum FN, Xi L, Kukreja RC. PDE5 inhibitors as therapeutics for heart disease, diabetes and cancer. *Pharmacol Therapeut.* 2015;147:12–21.
21. Ardehali H, O'Rourke B. Mitochondrial K(ATP) channels in cell survival and death. *J Mol Cell Cardiol.* 2005;39(1):7–16.
22. Korkmaz-Icoz S, Radovits T, Szabo G. Targeting phosphodiesterase 5 as a therapeutic option against myocardial ischaemia/reperfusion injury and for treating heart failure. *Brit J Pharmacol.* 2018;175(2):223–31.
23. Erol B, Tokgoz H, Hanci V, Bektas S, Akduman B, Yencilek F, et al. Vardenafil reduces testicular damage following ischemia/reperfusion injury in rats. *Kaohsiung J Med Sci.* 2009;25(7):374–80.
24. Ozgur BC, Telli O, Yuceturk CN, Sarici H, Ozer E, Surer H, et al. The effect of sildenafil and udenafil on testicular damage following ischemia-reperfusion injury in rats. *J Urol.* 2014;192(4):1272–7.
25. Ozmerdiven G, Coskun B, Kaygisiz O, Vuruskan BA, Asiltas B, Kilicarslan H. The protective effect of L-arginine, tadalafil, and their combination in rat testes after ischemia and reperfusion injury. *Cuaj-Can Urol Assoc.* 2017;11(1–2):E19–25.
26. Ameli M, Hashemi MS, Moghimian M, Shokoohi M. Protective effect of tadalafil and verapamil on testicular function and oxidative stress after torsion/detorsion in adult male rat. *Andrologia.* 2018;50(8):e13068.
27. Yildirim C, Yuksel OH, Urkmez A, Sahin A, Somay A, Verit A. Protective effects of Tadalafil and darbepoetin against ischemia - reperfusion injury in a rat testicular torsion model. *Int Braz J Urol.* 2018;44(5):1005–13.

28. Istanbulluoglu MO, Zor M, Celik A, Cicek T, Basal S, Ozgok A, et al. Effects of vardenafil on testicular torsion/detorsion damage: an experimental study in pigs. *Urol Int.* 2011;86(2):228–32.
29. Yildiz H, Durmus AS, Simsek H, Yaman I. Effects of sildenafil citrate on torsion/detorsion-induced changes in red blood cell and plasma lipid peroxidation, antioxidants, and blood hematology of male rats. *Eur J Obstet Gynecol Reprod Biol.* 2011;159(2):359–63.
30. Sengupta P. The Laboratory Rat: Relating Its Age With Human's. *Int J Prev Med.* 2013;4(6):624–30.
31. Bo Mi K, So Young C, Eun Hye L, Bo Hyun Y, Yun-Sok H, Bum Soo K et al, editors. Establishment and characterization of a rat model of testicular torsion. *KSBMB International Conference 2019; 2019; ICC JEJU, KOREA.*
32. Johnsen SG. Testicular biopsy score count—a method for registration of spermatogenesis in human testes: normal values and results in 335 hypogonadal males. *Hormones.* 1970;1(1):2–25.
33. Bartsch G, Frank S, Marberger H, Mikuz G. Testicular torsion: late results with special regard to fertility and endocrine function. *J Urol.* 1980;124(3):375–8.
34. Minutoli L, Irrera N, Squadrito F, Marini H, Nicotina PA, Arena S, et al. Effects of ischaemic post-conditioning on the early and late testicular damage after experimental testis ischaemia-reperfusion. *Andrology.* 2014;2(1):76–82.
35. Shirazi M, Noorafshan A, Karbalay-Doust S, Ardeshiri M, Afrasiabi MA, Monabati A. Comparison of the protective effects of papaverine, lidocaine and verapamil on the sperm quality of the testis after induced torsion-detorsion in rats. *Scand J Urol Nephrol.* 2010;44(3):133–7.
36. Shimizu S, Saito M, Kinoshita Y, Shomori K, Satoh I, Satoh K. Ischemic preconditioning and post-conditioning to decrease testicular torsion-detorsion injury. *J Urol.* 2009;182(4):1637–43.
37. Shimizu S, Tsounapi P, Dimitriadis F, Higashi Y, Shimizu T, Saito M. Testicular torsion-detorsion and potential therapeutic treatments: A possible role for ischemic postconditioning. *Int J Urol.* 2016;23(6):454–63.
38. Unsal A, Devrim E, Guven C, Eroglu M, Durak I, Bozoklu A, et al. Propofol attenuates reperfusion injury after testicular torsion and detorsion. *World J Urol.* 2004;22(6):461–5.
39. Ustun H, Akgul KT, Ayyildiz A, Yagmurdu H, Nuhoglu B, Karaguzel E, et al. Effect of phosphodiesterase 5 inhibitors on apoptosis and nitric oxide synthases in testis torsion: an experimental study. *Pediatr Surg Int.* 2008;24(2):205–11.
40. Uz E, Sogut S, Sahin S, Var A, Ozyurt H, Gulec M, et al. The protective role of caffeic acid phenethyl ester (CAPE) on testicular tissue after testicular torsion and detorsion. *World J Urol.* 2002;20(4):264–70.
41. Yang S, Shih HJ, Chow YC, Tsai PS, Wang TY, Wang PS, et al. The protective role of heme oxygenase-1 induction on testicular tissues after testicular torsion and detorsion. *J Urol.* 2007;177(5):1928–33.
42. Wu ZG, Wang GB, Xiao YB, Chen TK, Cai J, Li CD. [Protective effect of tadalafil against ischemia-reperfusion injury in rats]. *Zhonghua Nan Ke Xue.* 2015;21(3):214–8.

43. Jiang D, Wu D, Zhang Y, Xu B, Sun X, Li Z. Protective effects of hydrogen rich saline solution on experimental testicular ischemia-reperfusion injury in rats. *J Urol*. 2012;187(6):2249–53.
44. Mogilner JG, Lurie M, Coran AG, Nativ O, Shiloni E, Sukhotnik I. Effect of diclofenac on germ cell apoptosis following testicular ischemia-reperfusion injury in a rat. *Pediatr Surg Int*. 2006;22(1):99–105.
45. Beheshtian A, Salmasi AH, Payabvash S, Kiumehr S, Ghazinezami B, Rahimpour S, et al. Protective effects of sildenafil administration on testicular torsion/detorsion damage in rats. *World J Urol*. 2008;26(2):197–202.
46. Southorn PA, Powis G. Free radicals in medicine. I. Chemical nature and biologic reactions. *Mayo Clin Proc*. 1988;63(4):381-9.
47. Turner TT, Tung KS, Tomomasa H, Wilson LW. Acute testicular ischemia results in germ cell-specific apoptosis in the rat. *Biol Reprod*. 1997;57(6):1267–74.
48. Yasa H, Yakut N, Emreçan B, Ergunes K, Ortac R, Karahan N, et al. Protective effects of levosimendan and iloprost on lung injury induced by limb ischemia-reperfusion: a rabbit model. *J Surg Res*. 2008;147(1):138–42.
49. Okutan H, Savas C, Delibas N. The antioxidant effect of melatonin in lung injury after aortic occlusion-reperfusion. *Interact Cardiovasc Thorac Surg*. 2004;3(3):519–22.
50. Shimizu S, Saito M, Dimitriadis F, Kinoshita Y, Shomori K, Satoh I, et al. Protective effect of ischaemic post-conditioning on ipsilateral and contralateral testes after unilateral testicular ischaemia-reperfusion injury. *Int J Androl*. 2011;34(3):268–75.
51. Shiraishi K, Naito K, Yoshida K. Nitric oxide promotes germ cell necrosis in the delayed phase after experimental testicular torsion of rat. *Biol Reprod*. 2001;65(2):514–21.
52. Sarica K, Kupeli B, Budak M, Kosar A, Kavukcu M, Durak I, et al. Influence of experimental spermatic cord torsion on the contralateral testis in rats. Evaluation of tissue free oxygen radical scavenger enzyme levels. *Urol Int*. 1997;58(4):208–12.
53. Turner TT. Acute experimental testicular torsion. No effect on the contralateral testis. *J Androl*. 1985;6(1):65–72.
54. Madgar I, Lunenfeld B, Mashiach S, Goldwasser B, Weissenberg R. Effect of testicular torsion on contralateral testis and fertility in mature rats. *Arch Androl*. 1987;19(3):237–41.
55. Eid AA, Badr El Dine FM, Nabil IM. Histopathologic and Ultrastructural Changes in Seminiferous Tubules of Adult Male Albino Rats Following Daily Administration of Different Doses of Tadalafil. *Urology*. 2016;90:89–96.

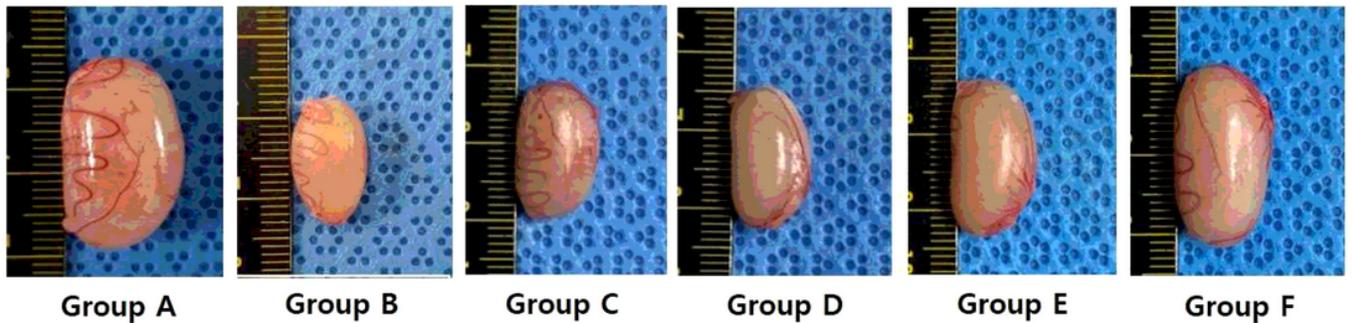
## Figures



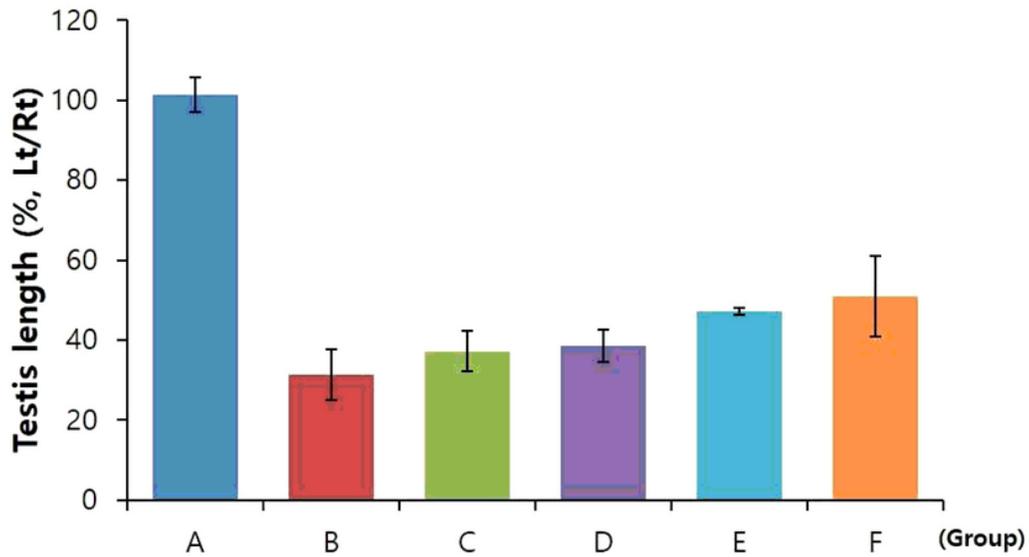
**Figure 1**

Experiment of testicular torsion. After left scrotal incision, unilateral testicular torsion was made in a 720° clockwise rotation on the left testis followed by the fixation to the scrotal wall with 4-0 silk suture.

(A)

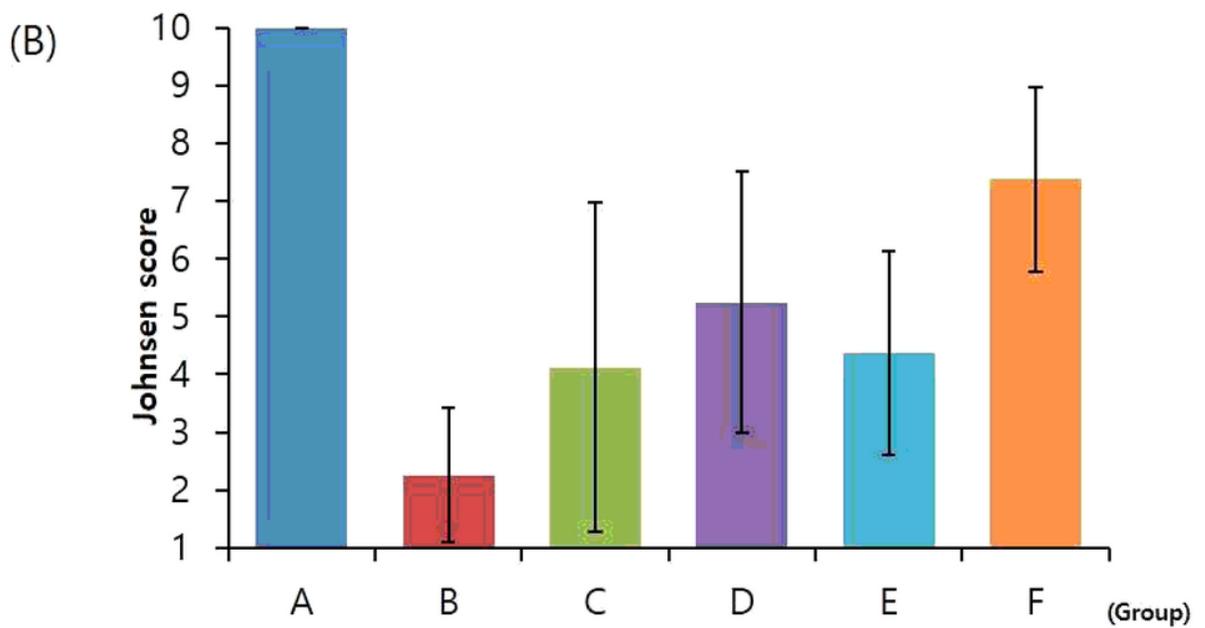
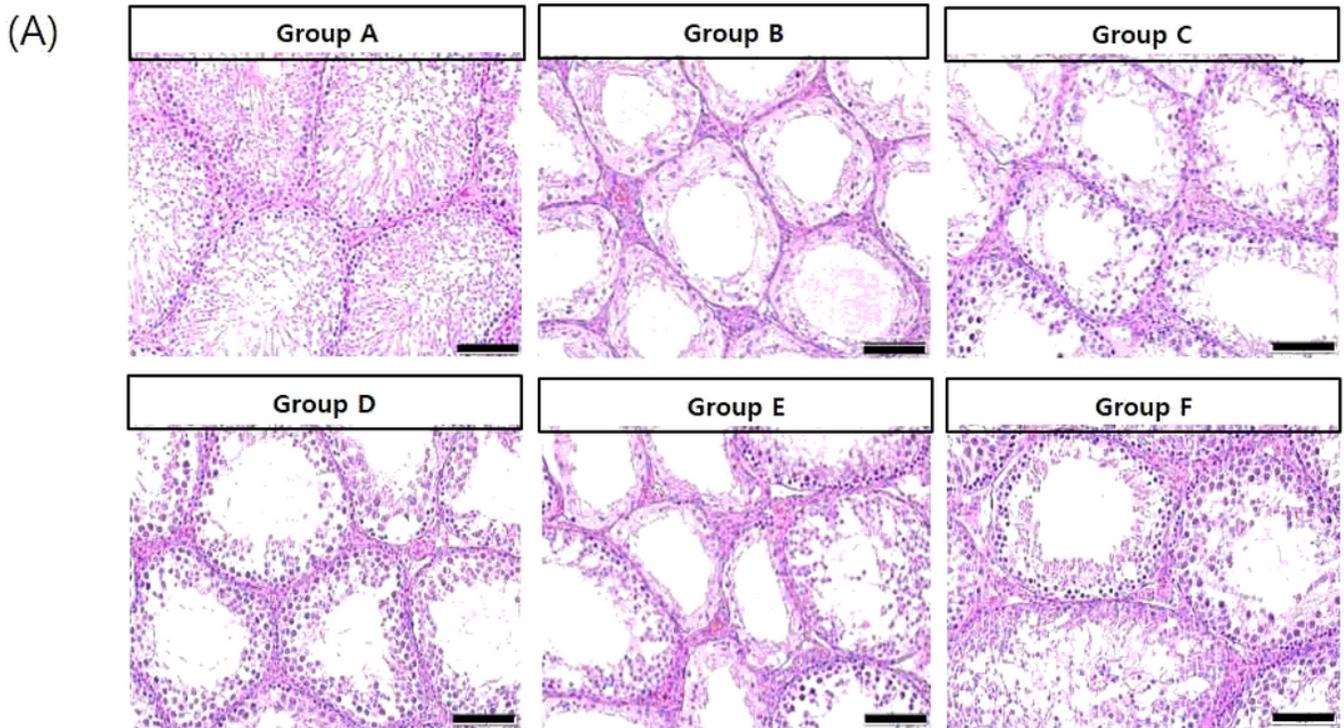


(B)



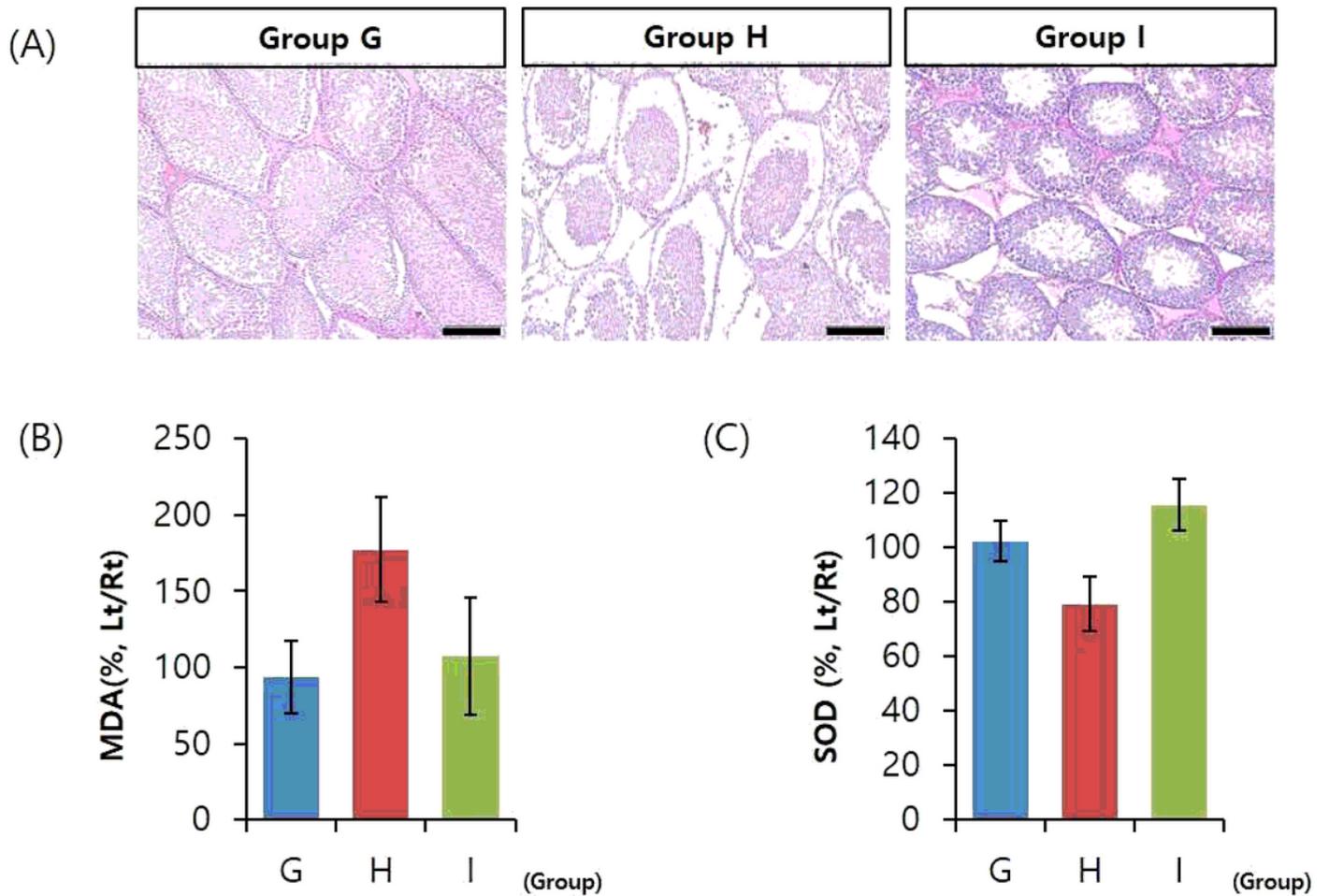
**Figure 2**

Representative gross images of the extracted left testes (A) and the mean value of maximal longitudinal length of the left testicle compared with that of the right (B) in groups A–F.



**Figure 3**

Representative histopathological images of left testes (A) and Johnsen scores (B) in groups A–F. Scale bars represent 100  $\mu\text{m}$  (magnification 200x)



**Figure 4**

Representative histopathological images of left testes (A) and molecular markers related with oxidative stress such as MDA (B) and SOD (C) in groups G–I. Scale bars represent 200  $\mu$ m (magnification 100x)

## Supplementary Files

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