Protective Effect of Udenafil Against Ischemia Reperfusion Injury Due to Testicular Torsion/Detorsion in Rat Model

Zulfu Sertkaya[1], Orhan Koca[2], Metin Ozturk[3], Mehmet Akyuz[3], Gulistan Gumrukcu[4], Musab Ali Kutluhan[5], Muhammet Ihsan Karaman[2]

Cite this article as: *Sertkaya Z, Koca O, Ozturk M, et al. Protective Effect of Udenafil Against Ischemia Reperfusion İnjury Due to Testicular Torsion/Detorsion in Rat Model. Eurasian J Med 2020; 52(2): 115-9.*

[1]Department of Urology, Memorial Hospital, Diyarbakir, Turkey
[2]Department of Urology, Medistate Hospital, Istanbul, Turkey
[3]Department of Urology, Haydarpasa Numune Training and Research Hospital, Istanbul, Turkey
[4]Department of Pathology, Haydarpasa Numune Training and Research Hospital, Istanbul, Turkey
[5]Department of Urology, Fatih Sultan Mehmet Training and Research Hospital, Istanbul, Turkey

Received: October 2, 2019
Accepted: September 29, 2019
Available Online Date: June 4, 2020

Correspondence to: Zulfu Sertkaya
E-mail: zlfsertkaya@gmail.com

DOI 10.5152/eurasianjmed.2020.19229

ABSTRACT

Objective: Testicular torsion causes migration of neutrophils to the ischemic region and formation of free oxygen radicals that have a critical effect on ischemic reperfusion (I/R) injury. Udenafil is a selective, strong, and reversible inhibitor of phosphodiesterase type enzyme. In our study, we evaluate the protective effect of udenafil against reperfusion injury due to I/R.

Materials and Methods: Twenty-one male, adult, Wistar-Albino rats aged 8 months were randomly divided into three groups; sham, I/R, and I/R+udenafil. One hour before the detorsion operation, the sham and I/R groupssaline, and I/R+udenafil groups were administered 2 mg/kg udenafil intraperitoneally. Blood samples were collected to evaluate the inflammatory mediators. Spermatogenic factors were evaluated according to Johnsen criteria.

Results: Histopathological and molecular parameters from all groups were compared. Mean values of TNF-α and IL-1β in venous blood samples were calculated. We observed that TNF-a values were statistically significantly increased in the I/R group than those in sham groups, and these values were decreased with udenafil treatment Furthermore, the glutathione peroxidase (GPx) level was statistically significantly decreased in the I/R group, and treatment with udenafil prevented this decrease. Evaluation of spermatogenesis using the Johnsen scoring system showed no statistically significant difference in mean scores between the groups.

Conclusion: We concluded that deterioration of biochemical and histopathological parameters are reversed, and injury due to I/R in testicle tissue may be decreased with udenafil treatment. Results of this experimental study show that efficacy of the udenafil treatment in testis torsion should be investigated.

Keywords: Ischemia-reperfusion, rat, testis, udenafil

Introduction

The torsion of testicle and spermatic cord are urologic emergencies in newborns, children, and adolescents. The incidence is about 1/4000 until 25 years old [1]. The survival rate of de-torsioned testes is about 48-88% [2]. However, spermatogenic functions are not well-known in these operated testes. It is indicated that a simple orchiectomy has to be done in late cases, which may cause infertility due to loss of function in the contralateral testes [3]. Although we do not clearly know how and when irreversible changes begin in the testes, intervention or detorsion surgery is recommended to be done in 4-6 h [4].

Testicular tissue is very sensitive to damage by free oxygen radicals, and germinal cells are seriously damaged by oxidative stress [5]. Studies show that 38% of males who have a history of torsion, have a sperm count of <20 million per mL [6]. Nitric oxide (NO) released from testis tissue and tumor necrosis factor (TNF)-α and interleukin (IL) 1-β released from neutrophils increase inflammation. Neutrophils play an important role in free oxygen radical formation by interacting with oxidative and nitrosative system. To determine the level of free oxygen radicals in tissue, the level of enzymes like glutathione peroxidase (GPx), nitric oxide sentetase (NOS), superoxide dismutase (SOD), and catalase which occur in these systems are calculated [7].

Tissue damage due to hypo-oxygenation in torsion can be reversed by immediate detorsion surgery. In addition, protective agents against reperfusion injury can provide more function in

testis after a detorsion surgery. For this reason, many protective agents have been investigated over years.

Phosphodiesterase 5 (PDE-5) enzyme is found intensively in the corpora cavernosa, the vascular and visceral smooth muscles and platelets [8]. PDE-5 inhibitors were used at first for the treatment of pulmonary hypertension, although they are widely used to treat erectile dysfunction [9]. Udenafil is one of the latest PDE-5 inhibitor to enter the markets and be used for the treatment of erectile dysfunction (ED). An increase in the level of cyclic guanosine monophosphate (cGMP) level in the tissue results in relaxation of smooth muscles. There are several studies about the effect of PDE-5 inhibitors (sildenafil, vardenafil, and tadalafil) on the ischemia-reperfusion injury due to testicular torsion [10-12]. In this study, we investigated the protective effect of udenafil on the ischemia-reperfusion injury due to testicular torsion like the other PDE-5 inhibitors.

Materials and Methods

The experimental protocol was approved by the Experimental Animal Center (DEHAMER) Ethics Committee. After approval, the animals were handled in compliance with the recommendations of the local animal care committee and the Principles of Laboratory Animal Care (NIH publication No. 85-23, revised 1985).

Twenty-one, male, adult Wistar-Albino rats, weighing 250-350 g, aged 8 months were randomized and divided into three groups; sham, I/R, and I/R + udenafil. Prior to the experiment, the subjects were habituated to laboratory conditions (22°C±2, 12 h light/12 h dark, 1 atmospheric pressure) and housed in dry cages for 2 weeks. Diet was city water and standard rat chow (Optima brand). Before induction of anesthesia prior to the procedure, the rats were weighed on 0.01-g precision scales and weights were recorded. After completion of all procedures including operation, while under general anesthesia, the subjects were decapitated.

For anesthesia, 10-mg/kg ketamine and 5-mg/kg xylazine (IP) were administered. During the operation, sterile conditions were provided for each rat. The left testis was separated from the gubernacular structure by midscrotal incision in all the rats. In the IR and IR+udenafil groups, the testis and cord elements were torsioned by rotating 720 degrees counter-clockwise. One hour before detorsion, 10-mg/kg saline (0.9% NaCl) was injected IP in the sham and IR groups; 2-mg/kg udenafil citrate (Zydena 100 mg, Zentiva) was given IP to the IR+udenafil group. During the second hour, detorsion procedure was applied. After 4 h of reperfusion, bilateral orchiectomies were performed, venous blood samples were taken, and the rats decapitated.

The orchiectomy materials coded according to the groups were brought to pathology laboratory after being fixed in 10% formalin. A 3-mm section was taken from each tissue and embedded in paraffin. After routine follow-up procedures, 3 micron sections were taken and stained with H&E and evaluated under microscope. An average of 25 seminiferous tubulus diameters (micrometer) was calculated for each tissue. In addition, scoring from 1 to 10 (Johnsen score) from bad to good was performed on the basis of 25 tubules per tissue for maturation of spermatogenesis.

Glutathione peroxidase levels in tissues were determined by immunohistochemical method. Three micron sections which were taken into polylyzine coated slides ready for immunohistochemical study, were incubated in an oven at 37 degrees overnight. After deparaffinization processes (15 minutes, 3 xylene, 5 minutes, 3 alcohol), immunohistochemical procedure was applied and the GPX-1 antibody was used for instillation (GPX-1, glutathione peroxidase, rabbit monoclonal antibody (EPR3312), GeneTex, Catolog No: GTX62554). Immunohistochemical examination was performed on human kidney tissue as a positive control. Immunohistochemical staining results were evaluated in accordance with a predetermined system. Accordingly, the intensity of cytoplasmic staining and the ratio of positive cells were determined. Expression score was determined by multiplying two components.

TNF-α and IL-1ß levels were measured in venous blood taken in tubes containing EDTA. Tissue damage was assessed by histopathological evaluation. Tissue GPx levels were determined by immunohistochemical methods. To evaluate the spermatogenic functions, each testis were scored according to the Johnsen criteria.

Statistical Analysis

Statistical Package for Social Science (IBM SPSS Corp.; Armonk, NY, USA) for Windows v.18.0 program was used for statistical evaluation. The distribution of data was analysed with the Shapiro-Wilk test. Student's t-test was used to compare continuous parametric variables, and the Mann-Whitney U test was used to compare continuous nonparametric variables. All numerical, nominal, and ordinal data obtained were compared with the Mann-Whitney U test and Dunn's test. Kruskal-Wallis test was used for comparison of multiple groups, and $p < 0.05$ was considered statistically significant.

Results

The average weight of rats included in study was 331.64±29.45 g. These averages were similar in all three groups, and there was no statistically significant difference between them. Average weight of the orchiectomy specimens extracted from mouse testis was similar between groups. The mean weights of the testes were 1.55, 1.62, and 1.66 in sham, I/R, and I/R+udenafil groups, respectively (Table 1).

Histopathological and molecular parameters from all the groups were compared after predicting torsion–detorsion times. Mean values of inflammatory mediators (TNF-α and IL-1β) in venous blood samples were calculated.

When I/R group was compared to the sham group, significantly higher levels of TNF-α were found (p=0.034). We observed that udenafil treatment given during reperfusion (I/R+U) significantly decreased the levels of TNF-α compared to that of the untreated I/R group (p=0.010). The TNF-α values were decreased with the udenafil treatment after I/R.

When we compared levels of expression score of GPx, we found that GPx of positively stained

Main Points

- I/R as a result of torsion–detorsion increased inflammatory mediators in the blood, GPx activity, decreased antioxidant capacity in tissue.
- I/R had no influence on spermatogenesis parameters for 4 h.
- The damage due to I/R can be prevented by udenafil treatment after I/R.

Table 1. Evaluation of parameters for each group

	n	Rat weight (average ± SD) (g)	Testis weight (average±SD) (g)	Tubulus diameter (μm)
Sham	7	329.19±24.76	1.55±0.07	318.47±33.67
I/R	7	323.57±45.41	1.62±0.12	282.01±23.65
I/R + U	7	342.15±25.44	1.66±0.14	250.41±17.52

I/R: ischemia-reperfusion, U: udenafil, g: gram, SD: standard deviation, μm: micrometer

Table 2. Comparison of GPx expression score averages

	n	Extent (average ± SD)	Intensity (average±SD)	Expression score (average±SD)
Sham (ipsilateral)	7	1.44±0.48	1.85±0.41	2.45±0.40
Sham (contralateral)	7	1.27±0.42	1.54±0.52	2.11±1.32
I/R (ipsilateral)	7	1.88±0.39	1.82±0.73	3.37±1.45
I/R (contralateral)	7	1.44±0.52	1.55±0.49	2.24±1.24
I/R + U (ipsilateral)	7	1.32±0.44	1.85±0.39	2.38±1.16
I/R + U (contralateral)	7	1.15±0.38	1.86±0.37	2.14±0.86

p<0.05, sham-I/R and I/R-I/R+V groups (ipsilateral); p>0.05, sham-I/R, Sham-I/R+V and I/R-I/R+V groups (contralateral);GPx: glutation peroxidase, I/R: ischemia-reperfusion, U: udenafil, SD: standard deviation, µm: micrometer

Table 3. Johnsen score averages after 4 h reperfusion

	n	Ischemic testis (average±SD)	Contralateral testis (average±SD)	Ischemic testis z value*	Contralateral testis z value*
Sham	7	9.3±0.1	9.3±0.2	0.0000	0.0000
I/R	7	9.1±0.2	9.2±0.2	0.9245	0.2145
I/R + U	7	9.2±0.2	8.7±0.3	0.6218	0.5517

*Kruskal Wallis Multiple Comparison Z-Value Test, comparison with sham group (when z >1.96 statistically significant); I/R: ischemia-reperfusion, U: udenafil, SD: standard deviation

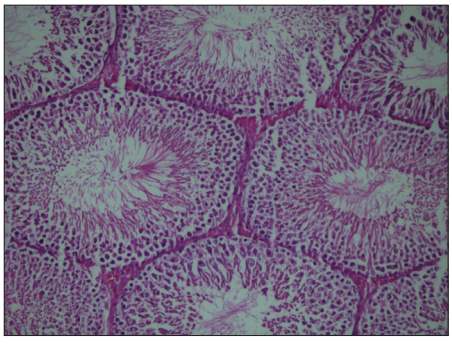

Figure 1. Seminiferous tubules in the regular structure, Sham, HEx200

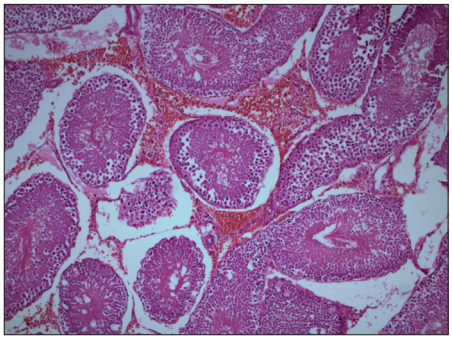

Figure 2. Hemorrhage in the interstitium, IR+Udenafil, HEx200

cells during reperfusion in the group administered udenafil (I/R+U) was statistically significant (p<0.05) as compared to those of the ischemia reperfusion (I/R) group. Moreover, similar levels of GPx scores were found in both the I/R + udenafil and the sham groups. Considering the prevalence of GPx scores between groups, there were no statistically significant difference

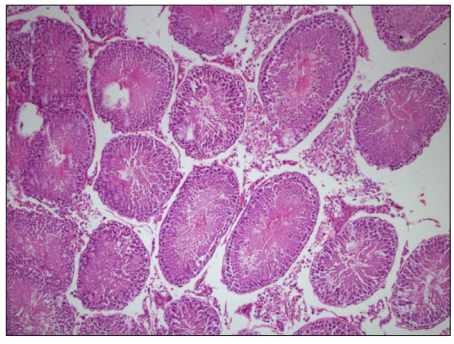

Figure 3. Edema and inflammatory cell accumulation in the interstitium, HEx200

(p>0.05). When GPx expression scores were evaluated, mean score of the I/R group was found to be significantly higher as compared to that of the sham group. Udenafil treatment decreased the levels of GPx significantly (p<0.05) (Table 2) (Figure 1, 2, 3).

In hematoxylin & eosin sections, seminiferous tubules and interstitium were regular in the sham group (Figure 1). Intestinal edema was observed in focal areas. In the ipsilateral testis in the IR group, the seminiferous tubules generally maintained their regular structure, but edema and inflammatory cell accumulation in the interstitium were remarkable and occasionally accompanied by interstitial hemorrhage foci (Figure 2). In the IR+udenafil group, significant edema and hemorrhage foci were observed in the ipsilateral testis compared to the sham group but was found to be lesser than that in the IR group (Figure 3).

Evaluation of spermatogenesis using the Johnsen scoring system showed that both testes, seminiferous tubules, and interstitial area were normal in the sham group. In the I/R group, the Johnsen score did not change significantly when compared with that of the sham group. When I/R+udenafil and sham groups were compared, udenafil treatment did not affect spermatogenesis and testicular parameters, it does not cause any increase (p>0.05).

Discussion

Testicular torsion is a urologic surgical emergency that affects mostly adolescents and young men. The incidence was determined as 1 in 4000 up to 25 years of age [1]. The experimental model of the torsion–detorsion on the testicle is a process of I/R injury, and germ cells are the most sensitive to I/R injury. By the tissue hypoxia and the following oxidative stress due to reperfusion, germ cells undergo an irreversible apoptotic process. A large number of mediators and cytokines take part in this process [13, 14].

Similar studies that use the I/R injury model have reported that damage occurs not only in the testes, but also in the contralateral testes in the presence of the drugs and chemicals used. Destruction of blood-testis barrier, autoimmune mediators against spermatogonium, increase in the production of ROS, and decrease in tissue perfusion by reflexive vasoconstruction with the sympathetic response may be implicated in the pathophysiology of the injury that occurred in the contralateral testis [15]. The protection of the contralateral testis continues to be of interest. There is no doubt that preservation of the non-torsioned contralateral testicle plays an important role in the fertility potential. While some studies have reported a low antioxidant capacity, high levels of lipid peroxidation, and high levels of histopathological damage to the contralateral testis, there are also contradictory studies that claim no significant damage to the contralateral testis [16, 17]. In our study, the level of histopathological damage and immunohistochemistry levels of the glutathione peroxidase in the contralateral testes, especially in the I/R group were found to be significantly higher than the sham group.

The efficacy of various drugs and chemical agents to minimize germ cell damage following an early detorsion surgery after testicular torsion and the contribution of torsion to infertility have been reported in numerous studies; however, very few of these studies have been applied to clinical practice because of their tolerability of side-effect profiles [18, 19].

Recently, several studies used PDE5 inhibitors, sildenafil, tadalafil, and vardenafil, against testicular reperfusion injury due to torsion–detorsion, but these studies are contradictory. Beheshtian et al. referred to the protective effects of low and high dose of sildenafil administered before detorsion, based on the increased levels of catalase and glutation peroxidase, decreased germ cell apoptosis, and lipid found peroxidation in their experiments [19]. Yıldırım et al. [20] investigated the protective effect of tadalafil and darbopoetin against ischemia reperfusion injury and concluded that the active forms of tadalafil, darbopoetin, and tadalafil/darbopoetin combination decreased histopathologic damage, caspase-3 activity, fibrosis score, and protected the torsioned and the contralateral testicle from reperfusion injury. Erol et al. [10] studied vardenafil against the reperfusion injury and found that the apoptosis protease activating factor 1, endothelial NOS, and inducible NOS levels were significantly reduced by vardenafil treatment. Ozgur et al. [21] conducted an experiment with udenafil and sildenafil, and it was the first study that utilized udenafil against reperfusion injury. They concluded that udenafil and sildenafil have a protective effect against reperfusion injury by reducing malondialdehyde levels and decreasing the oxidative stress. We evaluated the effect of udenafil on TNF-α and IL-1ß levels measured in venous blood and GPx levels in tissue by immunohistochemical methods. Furthermore, each testis were scored according to the Johnsen criteria to evaluate the spermatogenic function. In contrast, the study by Ustun et al. [18] reported that sildenafil and vardenafil did not have any protective effect against reperfusion injury in rat testicular torsion models.

Udenafil has already been studied in rat experimental models in the last decade. Yang et al. [22] studied the nephroprotective effect of udenafil against cyclosporin A-induced nephrotoxicity and concluded that udenafil and nitroprusside ameliorated renal injury associated with decreasing tubular apoptosis by decreasing eNOS and increasing VEGF in this model. There is another study about the renoprotective effect of udenafil against renal ischemia-reperfusion injury [23]. We found two studies about the protective effect of udenafil on testicular torsion model in rats. The first study was by Ozgur in 2014 [21], and the second study by Tuglu et al. [24] evaluated udenafil, piracetam, and dexmedetomidine in rat models. Udenafil was given at two different doses, i.e., 1.4 and 2.8 mg/kg intraperitoneally. They reported that the increasing doses of udenafil demonstrated antioxidant properties on the testis tissue and histopathologically protected the testicles. In our study, udenafil was given at a dose of 2 mg/kg and supported the protective effect of udenafil against reperfusion injury as shown in previous studies.

This study has several limitations. First, this is an animal study and a first step in terms of giving us an idea about an experimental drug or a treatment. Second, the number of rats used is very low, but it is not true to study with high numbers contrary to the animal rights.

In conclusion, I/R as a result of torsion–detorsion increased inflammatory mediators in the blood, increased GPx activity, decreased antioxidant capacity in tissue, and had no influence on spermatogenesis parameters for 4 h. We can conclude that the deterioration of biochemical and histopathological parameters can be reversed, and the damage due to I/R can be decreased by udenafil treatment. Results of this experimental study proves that efficacy of udenafil treatment in testis torsion should be studied.

Ethics Committee Approval: Ethics committee approval was received for this study Marmara University (DEHAMER).

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – Z.S., O.K., M.I.O.; Design - Z.S., M.A.; Supervision - M.I.K., M.I.O.; Resources - Z.S., M.A.K., G.G.; Materials - Z.S., G.G., M.A.; Data Collection and/or Processing - Z.S., M.A.K.; Analysis and/or Interpretation – O.K.; Literature Search - Z.S., M.A.; Writing Manuscript - Z.S., O.K., G.G.; Critical Review - M.I.K.

Conflict of Interest: Authors have no conflicts of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

References

1. Barada JH, Weingarten JL, Cromie WJ. Testicular salvage and age-related delay in the presentation of testicular torsion. J Urol 1989; 142: 746-8. [Crossref]
2. Cattolica EV, Karol JB, Rankin KN, et al. High testicular salvage rate in torsiion of the spermatic cord. J Urol 1982; 128: 66-8. [Crossref]
3. Mogilner JG, Lurie M, Coran AG, et al. Effect of diclofenac on germ cell apoptosis following ischemia-reperfusion injury in a rat. Pediatr Surg Int 2006; 22: 99-105. [Crossref]
4. Pentyala S, Lee J, Yalamanchili P, et al. Testicular torsion. A review. J Low Urin Tract Dis 2001; 5: 38-47. [Crossref]
5. Filho DW, Torres MA, Bordin AL, et al. Spermatic cord torsion, reactive oxygen and nitrogen species and ischemia-reperfusion injury. Mol Aspects Med 2004; 25: 199-210. [Crossref]
6. Visser AJ, Heyns CF. Testicular function after torsion of the spermatic cord. BJU Int 2003; 92: 200-3. [Crossref]
7. Jun P, Ko NU, English JD, et al. Endovascular Treatment of Medically Refractory Cerebral Vasospasm Following Aneurysmal Subarachnoid Hemorrhage. Am J Neuroradiol 2010; 31: 1911-6. [Crossref]
8. Francis SH, Turko IV, Corbin JD. Cyclic nucleotide phosphodiesterases: Relating structure and function. Prog Nucleic Acid Res Mol Biol 2001; 65: 1-52. [Crossref]
9. Sakuma M, Shirato K. [Phosphodiesterase type 5 inhibitors for pulmonary arterial hypertension]. Nihon Rinsho 2008; 66: 2157-61.
10. Erol B, Tokgoz H, Hanci V, et al. Vardenafil reduces testicular damage following ischemia/reperfusion injury in rats. Kaohsiung J Med Sci 2009; 25: 374-80. [Crossref]
11. Ameli M, Hashemi MS, Moghimian M, et al. Protective effect of tadalafil and verapamil on testicular function and oxidative stress after torsion/detorsion in adult male rat. Andrologia 2018; 50: e13068. [Crossref]
12. Kostakis ID, Zavras N, Damaskos C, et al. Erythropoietin and sildenafil protect gainst ischemia/reperfusion injury following testicular torsion in adult rats. Exp Ther Med 2017; 13: 3341-7. [Crossref]
13. Becker EJ Jr, Turner TT. Endocrine and exocrine effects of testicular torsion in the prepubertal and adult rat. J Androl 1995; 16: 342-51.
14. Cuzzocrea S, Riley DP, Caputi AP, et al. Antioxidant therapy: a new pharmacological approach in shock, inflammation, and ischemia/reperfusion injury. Pharmacol Rev 2001; 53: 135-59.
15. Sarica K, Kupeli B, Budak M, 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: 208-12. [Crossref]
16. Madgar I, Lunenfeld B, Mashiach S, et al. Effect of testicular torsion on contralateral testis and fertility in mature rats. Arch Androl 1987; 19: 237-41. [Crossref]
17. Özmerdiven G, Coskun B, Kaygisiz O, et al. The protective effect of L-arginine, tadalafil, and their combination in rat testes after ischemia and reperfusion injury. Can Urol Assoc J 2017; 11: E19-E25. [Crossref]
18. Ustun H, Akgul KT, Ayyildiz A, et al. Effect of phospodiesterase 5 inhibitors on apoptosis and nitric oxide synthases in testis torsion: an experimental study. Pediatr Surg Int 2008; 24: 205-11. [Crossref]
19. Beheshtian A, Salmasi AH, Payabvash S, et al. Protective effects of sildenafil administration on testicular torsion/detorsion damage in rats. World J Urol 2008; 26: 197-202. [Crossref]
20. Yildirim C, Yuksel OH, Urkmez A, et al. Protective effects of Tadalafil and darbepoetin against ischemia - reperfusion injury in a rat testicular torsion model. Int Braz J Urol 2018; 44: 1005-13. [Crossref]
21. Ozgur BC, Telli O, Yuceturk CN, et al. The Effect of Sildenafil and Udenafil to the Testicular Damage Following Ischemia/Reperfusion Injury in Rats J Urol. 2014; 192: 1272-7. [Crossref]

22. Yang JW, Kim JS, Kim MK, et al. The renoprotective effect of cGMP phosphodiesterase inhibitor and nitroprusside in a rat model of cyclosporin A-induced nephrotoxicity. Clin Nephrol 2013; 80: 53-62. [Crossref]

23. Özlülerden Y, Toktaş C, Aybek H, et al. The renoprotective effects of mannitol and udenafil in renal ischemia-reperfusion injury model. Investig Clin Urol 2017; 58: 289-95. [Crossref]

24. Tuglu D, Yuvanc E, Ozan T, et al. Protective effects of udenafil citrate, piracetam and dexmedetomidine treatment on testiculartorsion/detorsion-induced ischaemia/reperfusion injury in rats. Andrologia 2016; 48: 676-82. [Crossref]