World J Urol (2009) 27:117–122
DOI 10.1007/s00345-008-0323-7

ORIGINAL ARTICLE

Protective effect of trapidil on long-term histologic damage in a rat model of testicular ischemia-reperfusion injury

Murat Bozlu · Deniz Acar · Selahittin Cayan · Savas Aktas · Ahmet Tunckiran

Received: 27 September 2007 / Accepted: 4 August 2008 / Published online: 2 September 2008

Abstract
Objectives Trapidil is an antianginal compound with a broad spectrum of pharmacological activities. In recent years, it has been used successfully to decrease ischemia-reperfusion injury in several organ systems. We evaluated the effect of trapidil on the long-term histologic damage in testicular ischemia-reperfusion injury.
Methods Adult male Wistar rats were divided into three groups of six rats each. One group underwent 2 h of testicular torsion; one received pretreatment with trapidil before detorsion; and one group underwent sham operation. All rats underwent bilateral orchiectomy 60 days after the experiment. The mean seminiferous tubular diameter, germinal epithelial cell thickness, and mean testicular biopsy score were determined by histological examination of each testis.
Results Testicular torsion–detorsion caused a significant decrease in the mean seminiferous tubular diameter, germinal epithelial cell thickness, and mean testicular biopsy score in the ipsilateral testes, but not in the contralateral testes. The animals treated with trapidil had a significant increase in these histological parameters as compared to the torsion–detorsion group.
Conclusion Trapidil administration before reperfusion may have the potential to decrease the long-term histologic damage that occurs after experimental testicular torsion. Trapidil is used as an antianginal drug and additional clinical studies are required to elucidate the protective role of trapidil in patients with testicular torsion.

Keywords Testis · Reperfusion injury · Trapidil · Rats

M. Bozlu (✉) · D. Acar · S. Cayan · A. Tunckiran
Department of Urology, University of Mersin School of Medicine, Zeytinlibahce Caddesi, 33079 Mersin, Turkey
e-mail: muratbozlu@yahoo.com

S. Aktas
Department of Histology/Embryology, University of Mersin School of Medicine, Mersin, Turkey

Introduction

Testicular torsion is a common urological emergency among newborns, children and adolescents. The salvage rate is directly proportional to the duration of torsion, and early diagnosis followed by detorsion is the current management for the preservation of spermatogenesis and fertility [1]. Although reperfusion is essential for the survival of ischemic tissue, there is good evidence that reperfusion itself causes the pathophysiological cascades including an activation of neutrophils, inflammatory cytokines, and adhesion molecules with increased thrombogenicity, release of massive intracellular Ca^{2+}, and generation of oxygen-derived free radicals [2, 3]. Reactive oxygen species cause DNA damage, endothelial damage, and germinal cell necrosis.

Trapidil [5-methyl-7-diethylamino-s-triazolo(1,5-α)pyrimidine], an inhibitor of phosphodiesterase and platelet-derived growth factor, is an antianginal drug with a broad spectrum of pharmacological activities [4]. Over the past decade, several studies have demonstrated the protective effect of trapidil in various forms of tissue injury including kidney, spinal cord, peripheral nerves, and small intestine [5–8]. More recently, it has been demonstrated that administration of trapidil before detorsion had a protective role in the early period of the testicular biochemical changes associated with ischemia/reperfusion (I/R) injury [9]. However, to our knowledge, the role of trapidil on the long-term histological damage in testicular I/R injury is undefined. In the present study, we examined whether trapidil has a protective effect

on long-term histologic damage after testicular torsion followed by 60 days recovery.

Materials and methods

The study included 18 adult male Wistar rats weighing 270–300 g. The rats were maintained on a 12-h light/dark cycle. The ethical committee on animal research at our institution approved the protocol for all animal experiments. We provided appropriate care and use of the laboratory animals as recommended by the Board of Registry publication guidelines.

Chemicals

We used an antianginal drug trapidil (Rocornal, Rentschler Biotechnologie GmbH, Laupheim, Germany). The administration mode and dose of trapidil in this study corresponded to those used in previous experimental studies [6, 9].

Animal preparation and surgical procedure

The rats were divided into three groups of six rats each. One group underwent 2 h of testicular torsion; the second received pretreatment with trapidil before detorsion; and the third underwent a sham operation. Surgery was done with the subject under ketamine anesthesia (single intraperitoneal 50-mg/kg dose). All surgical procedures were performed through standard ilioinguinal incisions [10–12].

In the torsion–detorsion (T–D) group, the gubernaculum was divided, and the testis was freed from its longitudinal and distal pole attachment to the epididymis. Torsion was created by rotating the left testis 720° clockwise and maintained by fixing the testis to the scrotum with a 4-0 silk suture placed through the tunica albuginea. After 2 h of torsion, the testis was counter-rotated back to the natural position and reinserted into the scrotum. The wound was closed using 3-0 catgut suture.

In the group receiving trapidil before detorsion, the same surgical procedure was done as in the T–D group, but trapidil (40 mg/kg) was injected intraperitoneally for 30 min before detorsion and closure.

In the control group (sham group), a sham procedure was performed. This consisted of same procedure as in the T–D group, except that after rotating the testis 720° clockwise, it was immediately relieved, and a 4-0 silk suture was placed through the tunica albuginea.

Histologic preparation

After 60 days, bilateral orchiectomy was performed, and the rats were killed by pentobarbital overdose (200 mg/kg) and bilateral thoracotomy. The testes were fixed in Bouin's solution (7.5 mL saturated picric acid, 2.65 mL glacial acetic acid and 2.5 mL 7% formaldehyde), postfixed in 70% alcohol, and embedded in paraffin blocks. Sections (5 μm) were obtained, deparaffinized, and stained with hematoxylin–eosin.

Histologic evaluation

The testicular tissue was evaluated in random order with standard light microscopy by an observer who was unaware as to which group the rat had belonged. Three slides, prepared from the upper, lower, and midportions of the testes, were evaluated completely for each testis. The mean seminiferous tubular diameter (MSTD), germinal epithelial cell thickness (GECT), and mean testicular biopsy score (MTBS) were used to evaluate in 20 seminiferous tubules of each section. The MSTD was calculated using a microscope-adaptable micrometer. The MSTD of each testis was determined in microns. GECT was determined by counting the number of epithelial cells from the basement membrane to the lumen at 90°, 180°, 270°, and 360°, and averaged. The MTBS was graded using Johnsen's score [13]. A score of 0–10 was given to each tubule according to epithelial maturation.

Statistical analysis

All data were expressed as the mean ± standard deviation. Analysis of variance was used for statistical analysis of the data among the groups. Multiple comparisons were made using Tukey's procedure, with $P < 0.05$ considered statistically significant.

Results

The values of MSTD, GECT, and MTBS of each group are shown in Table 1 and Fig. 1a–c. When compared with the sham group, the MSTD, GECT, and MTBS obtained from the T–D group were significantly lower in the ipsilateral testes ($P < 0.001$). These three histologic parameters in the ipsilateral testes of the trapidil group were significantly higher than the values in the T–D group ($P < 0.01$). All the parameters of contralateral testes did not reveal any statistically significant differences among these groups ($P > 0.05$).

Discussion

Testicular torsion is a surgical emergency. Late presentation or failure to diagnose and correctly manage this condition leads to testicular injury and subfertility [1]. The

Table 1 Mean values of seminiferous tubular diameter (MSTD), germinal epithelial cell thickness (GECT), and testicular biopsy score (MTBS) in ipsilateral and contralateral testes

Groups	MSTD ± SD (μm)		GECT (mean cell layers ± SD)		MTBS ± SD	
	Ipsilateral	Contralateral	Ipsilateral	Contralateral	Ipsilateral	Contralateral
Torsion–detorsion[a]	124.41 ± 22.45	264.14 ± 13.23	1.4 ± 0.43	7.92 ± 0.44	3.41 ± 1.45	9.46 ± 0.25
Trapidil[b]	180.24 ± 36.02	253.47 ± 11.91	4.41 ± 2.58	7.75 ± 0.93	7.65 ± 1.70	9.72 ± 0.14
Sham	281.17 ± 12.21	264.14 ± 13.23	8.07 ± 0.6	8.03 ± 0.66	9.90 ± 0.39	9.81 ± 0.70

[a] Versus sham in the ipsilateral testes ($P < 0.001$)

[b] Versus torsion–detorsion in the ipsilateral testes ($P < 0.01$)

No statistically significant differences in contralateral testes among groups ($P > 0.05$)

results of the present study indicate that testicular T–D induces progressive histologic changes as a result of I/R injury. Previous studies with a rat model of testicular torsion have demonstrated that a 2-h, 720° rotation of the testis followed by reperfusion causes a significant increase in testicular lipid peroxidation products, nitric oxide (NO) content, and neutrophil accumulation [10]. The events result in permanent loss of spermatogenesis. The lesions in the present study are characterized by a decrease in seminiferous tubule diameter, germinal epithelial cell thickness, and degree of spermatozoal maturation.

Trapidil is an antiplatelet agent that acts in part as a phosphodiesterase inhibitor and a competitive inhibitor of the platelet-derived growth factor. It has been used as a coronary agent in clinical practice for many years [4]. On the other hand, trapidil has the pharmacological properties including nitroglycerine-like vasodilating action, inhibition of platelet aggregation, facilitation of the biosynthesis of prostacyclin, inhibition of thromboxane A2 and reduction of lipid peroxidation [6, 9, 14]. It also reduces the production of tumor necrosis factor-alpha, interleukin-6, and interleukin-12 and procoagulant activity by inhibition of the CD40 pathway of monocytes and macrophages [15]. As trapidil has these broad spectrum of biological activities, several investigators used trapidil in various forms of tissue injury including kidney, spinal cord, peripheral nerves, and small intestine [5–8].

In the present study, we evaluated the effects of trapidil only in the late phase of I/R. More recently, the effects of trapidil in early phase of testicular I/R has also been reported [9]. Somuncu et al. [9] demonstrated that trapidil administration before 4 h of detorsion caused a statistically significant decrease in testicular malondialdehyde and NO levels in the rat model of testicular torsion. They concluded that trapidil decreased free oxygen radical formation in testicular torsion and detorsion. However, very short follow-up in that study was not adequate to determine the true protective effect of trapidil on histologic damage in testicular I/R injury. Somuncu et al. [9] found that no histologic changes were seen in sham and sham plus trapidil groups in the early phase of testicular I/R. The additional results may seem of little clinical relevance and we did not use sham plus trapidil group.

Interstitial changes related to I/R of the testis are important and Somuncu et al. [9] demonstrated that I/R creates increased capillary edema, congestion, interstitial hemorrhage and hemorrhagic infarcts in the injured testis. They also reported that trapidil reduced cellular damage and hemorrhage in testicular torsion and detorsion. The successful results in the early period led us to evaluate the effect of trapidil on long-term histologic damage in the testicular torsion model of the rat. In the present study, we have obtained data showing for the first time that trapidil is effective in reducing long-term histologic damage resulting from testicular I/R, and preservation of the histological parameters, including MSTD, GECT, and MTBS, are significantly maintained in animals treated with trapidil.

In the present study, we have examined three histological parameters associated with reperfusion injury: (1) seminiferous tubule diameter, (2) germinal epithelial cell thickness, and (3) degree of spermatozoal maturation. The results of our study support the hypothesis of improved seminiferous tubule diameter, survival of the germinal epithelium, and preservation of spermatogenesis with trapidil treatment before detorsion of the testis. All these histologic parameters may give an accurate measurement of the degree to which spermatozoal maturation is taking place within the seminiferous tubule and also the level of spermiogenesis that is related to fertility [11, 12]. However, several previous studies evaluated the sperm concentration and quality for long-term evaluation of testicular I/R injury [16–19]. We did not use sperm agglutination test, daily sperm production, and epididymal sperm concentration and motility. Our results may encourage other investigators to evaluate the effect of trapidil on sperm concentration and quality for long-term evaluation of testicular I/R injury in further studies.

Most of the previous experimental studies showed the beneficial effects of the reactive oxygen species scavengers on short-term results in testicular I/R injury. Can et al. [20]

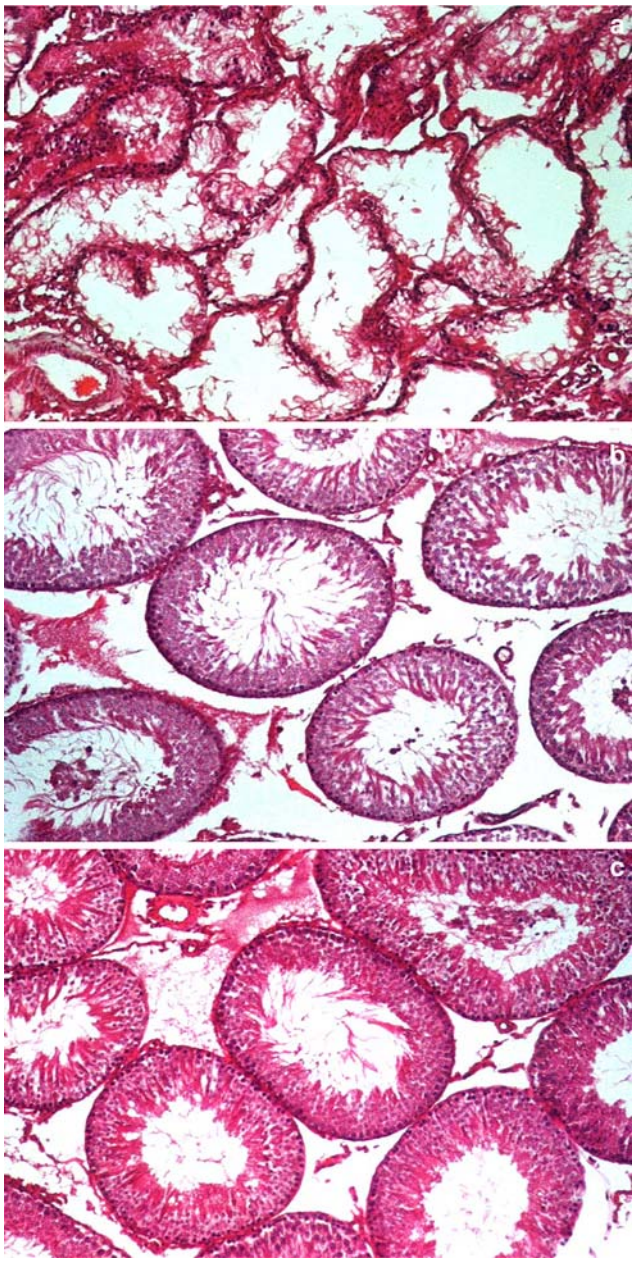

Fig. 1 Histologic findings of ipsilateral testes in (**a**) torsion–detorsion, (**b**) trapidil, and (**c**) sham groups. Note, no recovery of spermatogenesis observed in testes of torsion–detorsion group and preservation of spermatogenesis in trapidil group and normal spermatogenesis in sham group. H&E stain, reduced from ×100

reported that vasoactive intestinal peptide (VIP) can protect testicular tissue from 2 h of torsion followed by 1 h of detorsion. However, they found that the protective effect of VIP was attenuated in the 4-h detorsion. Only two studies to date reported long-term protective effects of antioxidants on testicular I/R injury [21, 22]. Although the treatment with antioxidants had palliative effect on the histologic changes in testes that underwent 1 h of experimental torsion, no significant rescue was seen after 2 h of testicular ischemia [21, 23]. In contrast, two recent studies from our institution using a rat model of 2 h testicular torsion have reported a significant rescue with the use of poly(adenosine diphosphate-ribose)polymerase inhibitors and vascular endothelial growth factor [11, 12]. In the present study, trapidil administration has also provided long-term protection for testicular I/R injury in a rat model.

In the present study, we found that trapidil provided a statistically significant decrease in long-term histologic damage after 2 h of testicular torsion followed by detorsion. The pathogenesis of testicular damage after I/R injury has multiple mechanisms and the protective effects of trapidil could not be explained by a simple antioxidant effect. As previously stated, trapidil treatment before detorsion significantly reduced the concentrations of testicular NO [9]. NO, a gaseous molecule with diverse biologic functions, is synthesized from L-arginine by a family of isoenzymes termed NO synthases (NOSs). The results obtained from previous studies suggested that NO played an important role in damaging the testis with I/R [9, 10]. Our previous study demonstrated that reperfusion for 4 h following 2 h of ischemia elevates NO production in the model of testicular torsion of rat [10].

On the other hand, it has been demonstrated that the loss of spermatogenesis after I/R of the testis is due to germ cell-specific apoptosis [24]. Reperfusing leukocytes are potent generators of reactive oxygen species, and the recruitment of neutrophils to the testis after torsion is essential for the observed pathology [3, 10, 24]. Extravasated neutrophils become activated once in the inflammatory sites, secreting a variety of substances such as growth factors, chemokines, and cytokines, complement components, proteases, NO, reactive oxygen metabolites, and peroxynitrite, all important mediators of tissue injury [2]. Inhibition of apoptosis may be important protective effect provided by trapidil. However, it has been reported that TNF receptors induce apoptosis, and trapidil reduces the production of TNF-α [15]. Further studies are required to clarify the relationship between trapidil and germ cell-specific apoptosis. Savas et al. [25] demonstrated that the fibrous tunica propria enveloping the seminiferous tubule was thickened due to increased collagen fibers in contralateral testis. They also found that the gap between basal lamina and the germ cells was increased because of collagen fibers. Though apoptotic damage is expected to resolve early after I/R, its long-term consequences may be examined in testes tissue long after torsion.

To date, there have been numerous experimental studies done into the role of several treatment modalities within testicular torsion [26–29]. However, none have been tested in clinical trials, apart from cooling the scrotum [1]. Trapidil has been used as an antianginal drug in humans, and it may have the clinical applicability in patients with torsion

of the testicle. The use of this drug in humans in both previous studies and clinically without significant side effects can make its potential use in testicular torsion more attractive. On the basis of the data presented in this study, we propose that administration of trapidil may be a novel approach for the therapy of I/R injury of the testis.

The effect of unilateral torsion on the contralateral testis has been controversial. It has been reported that ipsilateral torsion does not result in contralateral testicular damage in rats [30]. Our previous studies demonstrated that biochemical and histologic parameters of the contralateral testes did not reveal any statistical differences [10–12]. The results of the present study support our previous findings.

The results of the present study showed that trapidil administration before the reperfusion period of testicular torsion may result in prolonged testicular salvage. Trapidil treatment before reperfusion may have the potential to decrease the long-term histologic damage that occurs after testicular torsion. As this drug is used in humans to control angina pectoris or prevent platelet aggregation, we propose that trapidil may have the clinical applicability in patients with torsion of the testicle. For this purpose, further clinical studies will be needed.

Conflict of interest statement There is no conflict of interest.

References

1. Visser AJ, Heyns CF (2003) Testicular function after torsion of the spermatic cord. BJU Int 92:200–203
2. Cuzzocrea S, Riley DP, Caputi AP, Salvemini D (2001) Antioxidant therapy: a new pharmacological approach in shock, inflammation, and ischemia/reperfusion injury. Pharmacol Rev 53:135–159
3. Lysiak JJ, Nguyen QA, Kirby JL, Turner TT (2003) Ischemia-reperfusion of the murine testis stimulates the expression of proinflammatory cytokines and activation of c-jun N-terminal kinase in a pathway to E-selectin expression. Biol Reprod 69:202–210
4. Maresta A, Balducelli M, Cantini L, Casari A, Chioin R, Fabbri M, Fontanelli A, Monici Preti PA, Repetto S, De Servi S (1994) Trapidil (triazolopyrimidine), platelet-derived growth factor antagonist, reduced restenosis after percutaneous transluminal coronary angioplasty: Results of the randomized, double-blind STARC study. Circulation 90:2710–2715
5. Avlan D, Tamer L, Ayaz L, Polat A, Ozturk C, Ozturhan H, Camdeviren H, Aksoyek S (2006) Effects of trapidil on renal ischemia-reperfusion injury. J Pediatr Surg 41:1686–1693
6. Gocer AI, Ildan F, Tuna M, Polat S, Tamer L, Erman T, Kaya M (2001) Effects of trapidil on ATPase, lipid peroxidation, and correlation with ultrastructure in experimental spinal cord injury. Neurosurg Rev 24:136–142
7. Bagdatoglu C, Saray A, Surucu HS, Ozturk H, Tamer L (2002) Effect of trapidil in ischemia/reperfusion injury of peripheral nerves. Neurosurgery 51:212–220
8. Colak T, Polat A, Bagdatoglu O, Kanik A, Turkmenoglu O, Aydin S (2003) Effect of trapidil in ischemia/reperfusion injury on rat small intestine. J Invest Surg 16:167–176
9. Somuncu S, Cakmak M, Erdogan S, Caglayan O, Akman H, Kaya M (2006) Protective effects of trapidil in ischemia-reperfusion injury due to testicular torsion and detorsion: an experimental study. Int J Urol 13:601–605
10. Bozlu M, Eskandari G, Çayan S, Canpolat B, Akbay E, Atik U (2003) The effect of poly(adenosine diphosphate-ribose) polymerase inhibitors on biochemical changes in testicular ischemia-reperfusion injury. J Urol 169:1870–1873
11. Bozlu M, Coskun B, Cayan S, Acar D, Aktas S, Ulusoy E, Akbay E (2004) Inhibition of poly(adenosine diphosphate-ribose) polymerase decreases long-term histologic damage in testiscular ischemia-reperfusion injury. Urology 63:791–795
12. Tunckiran A, Cayan S, Bozlu M, Yilmaz N, Acar D, Akbay E (2005) Protective effect of vascular endothelial growth factor on histologic changes in testicular ischemia-reperfusion injury. Fertil Steril 84:468–473
13. Johnsen SG (1970) Testicular biopsy score count-A method for registration of spermatogenesis in human testis: normal values and result in 352 hypogonadal males. Hormones 1:2–25
14. Nieder J, Claus P, Auguntin W (1995) Effect of trapidil on the PGI_2 and TxA_2 synthesis in human umbilical veins perfused in vitro. Prostaglandins 49:311–318
15. Zhou L, Ismaili J, Stordeur P, Thielemans K, Goldman M, Pradier O (1999) Inhibition of the CD40 pathway of monocyte activation by triazolopyrimidine. Clin Immunol 93:232–238
16. Arap MA, Vicentini FC, Cocuzza M, Hallak J, Athayde K, Lucon AM, Arap S, Srougi M (2007) Late hormonal levels, semen parameters, and presence of antisperm antibodies in patients treated for testicular torsion. J Androl 28:528–532
17. Adivarekar PK, Bhagwat SS, Raghavan V, Bandivdekar AH (2005) Effect of Lomodex-MgSO(4) in the prevention of reperfusion injury following unilateral testicular torsion: an experimental study in rats. Pediatr Surg Int 21:184–190
18. Becker EJ Jr, Turner TT (1995) Endocrine and exocrine effects of testicular torsion in the prepubertal and adult rat. J Androl 16:342–351
19. Payabvash S, Kiumehr S, Tavangar SM, Dehpour AR (2008) Ethyl pyruvate reduces germ cell-specific apoptosis and oxidative stress in rat model of testicular torsion/detorsion. J Pediatr Surg 43:705–712
20. Can C, Tore F, Tuncel N, Uysal O, Gurer F, Ak D, Tuncel M (2004) Protective effect of vasoactive intestinal peptide on testicular torsion-detorsion injury: association with heparin-containing mast cells. Urology 63:195–200
21. Prillaman HM, Turner TT (1997) Rescue of testicular function after acute experimental torsion. J Urol 157:340–345
22. Kehinde EO, Anim JT, Mojiminiyi OA, Al-Awadi F, Shihab-Eldeen A, Omu AE, Fatinikun T, Prasad A, Abraham M (2005) Allopurinol provides long-term protection for experimentally induced testicular torsion in a rabbit model. BJU Int 96:175–180
23. Lysiak JJ, Nguyen QA, Turner TT (2002) Peptide and nonpeptide reactive oxygen scavengers provide partial rescue of the testis after torsion. J Androl 23:400–409
24. Turner TT, Tung KS, Tomomasa H, Wilson LW (1997) Acute testicular ischemia results in germ cell-specific apoptosis in the rat. Biol Reprod 57:1267–1274
25. Savas C, Ozogul C, Karaoz E, Bezir M (2002) Ischemia, whether from ligation or torsion, causes ultrastructural changes on the contralateral testis. Scand J Urol Nephrol 36:302–306
26. Uz E, Sogut S, Sahin S, Var A, Ozyurt H, Gulec M, Akyol O (2002) The protective role of caffeic acid phenethyl ester (CAPE) on testicular tissue after testicular torsion and detorsion. World J Urol 20:264–270
27. Unsal A, Devrim E, Guven C, Eroglu M, Durak I, Bozoklu A, Balbay MD (2004) Propofol attenuates reperfusion injury after testicular torsion and detorsion. World J Urol 22:461–465

28. Yazihan N, Ataoglu H, Koku N, Erdemli E, Sargin AK (2007) Protective role of erythropoietin during testicular torsion of the rats. World J Urol 25:531–536
29. Beheshtian A, Salmasi AH, Payabvash S, Kiumehr S, Ghazinezami B, Rahimpour S, Tavangar SM, Dehpour AR (2008) Protective effects of sildenafil administration on testicular torsion/detorsion damage in rats. World J Urol 26:197–202
30. Turner TT (1987) On unilateral testicular and epidymal torsion: no effect on the contralateral testis. J Urol 138:1285–1290