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Role of antioxidants in preventing testicular ischemia-reperfusion injury: a narrative review

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Abstract. – Testicular ischemia-reperfusion injury (TIR) is a urological emergency common among male newborns, children, and adolescents. Testicular injury and its consequences, such as altered hormone production, subfertility, and infertility, are determined by the duration and degree of testicular torsion. Early diagnosis and treatment are crucial to preserving the testes and fertility. Previous studies suggest that reactive oxygen species contribute to the pathogenesis of TIR injury, but the underlying mechanism remains unclear. Several drugs/plants reportedly exhibit antioxidative activities to protect against TIR. This review summarizes current studies on the role of antioxidants in preventing experimental TIR injury and discusses the underlying pathophysiological mechanisms.

Key Words:
Testicular ischemia-reperfusion injury, Reactive oxygen species, Antioxidants.

Abbreviations

Testicular ischemia-reperfusion injury (TIR); Reactive oxygen species (ROS); Testicular torsion and detorsion (TTD); Ratio of bilateral testicular weight (RBTW); Superoxide dismutase (SOD); Catalase (CAT); Glutathione peroxidase (GPx); Malondialdehyde (MDA); Myeloperoxidase (MPO); Interleukin-1 beta (IL-1β); Tumor necrosis factor alpha (TNF-α); Mean seminiferous tubule diameter (MSTD); Total oxidative capacity (TOC); Oxidative stress index (OSI); Total antioxidant status (TAS); Total oxidant status (TOS); Nuclear factor kappa B (NF-κB); Endothelial nitric oxide synthase (eNOS); Johnsen scores (JS); Proliferating cell nuclear antigen (PCNA); Nitric oxide synthase (NOS); Xanthine oxidase (XO).

Introduction

Testicular ischemia-reperfusion (TIR) injury results from testicular torsion/detorsion (TTD)[1], a urological emergency common among male newborns, children, and adolescents[2,3]. Its incidence is 1 out of 4,000 males below 25 years of age, and approximately 50% of them suffer from testicular atrophy and dysfunction despite successful surgical detorsion[4,5]. Testicular injury and its consequences, such as altered hormone production, subfertility, and infertility, are determined by the duration and degree of torsion[6]. Early diagnosis and treatment are crucial to preserve the testes and fertility because the testis suffers significant ischemic damage after 4-8 h[7,8]. Testicular torsion is fixed through surgery, and involves the detorsion of testicular torsion and the restoration of testicular blood flow[9]. Apart from ischemia, reperfusion after surgical testicular detorsion is also involved in the pathophysiological changes within the affected testis[10].

The pathological mechanisms underlying TIR injury remain unclear to date[11]. Notably, mammalian testes are highly sensitive to oxidative stress because their cell membranes contain high levels of polyunsaturated fatty acids[3]. Another reason is their constantly active cell division and spermatogenesis[12]. Consequently, testicular cells consume large amounts of oxygen and are extremely vulnerable to oxygen depletion[13]. Oxidative status imbalance results in TIR[5,14]. Free radicals such as superoxide, hydroxyl, and peroxynitrite accumulate during ischemia and partly explain the injury after ischemia[15]. Reactive oxygen species (ROS) production damages tissue by inducing cell-membrane lipid peroxidation, protein denaturation, and DNA impairment[2,5,16]. Antioxidants are the first line of defense of organisms against the harmful effects of TIR injury on testicular cell[17].

This review summarizes current studies on the protective role of antioxidants in TIR injury and discusses the pathophysiological changes that occur during TIR injury. Possible translation from experimental studies to clinical practice is also presented.

Performance of TIR

The torsion must be treated promptly to avoid loss of function of the ipsilateral and contralateral

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testis. This syndrome often leads to infertility of the ipsilateral (torted) and contralateral (not torted) testis, but the effect of TIR on the contralateral testis remains controversial to date. Some studies[5,18,19] have reported that unilateral TIR damage the contralateral testis[18,19], whereas this phenomenon has not been observed in another study[5]. Dejban et al[20] showed that unilateral testicular torsion of the ipsilateral testis at 720° for 1 h and after 7 days of reperfusion inflicts biochemical and histological damages to the contralateral testis. Xiao et al[21] proved that the mean volume of the ipsilateral testis in the ischemia-reperfusion (I/R) injury group was significantly larger than that of the contralateral testis after spermatic-cord ligation. Xanthine oxidase protein expression, malondialdehyde (MDA) levels, and spermatogenesis in the ipsilateral testes significantly changed after unilateral TIR, but those in contralateral testes showed no significant changes[5]. These irreversible changes can be associated with a subsequent reduction in fertility[22].

Testicular torsion is a urological syndrome caused primarily by a twist in the spermatic cord. Surgical detorsion should be performed immediately to prevent loss of function in the ipsilateral testes. A previous study[23] has showed that the testicular weight of the TTD group was significantly less than that of the control group. Sugiyama et al[24] evaluated the degree of atrophy of the ipsilateral testis by calculating the ratio of bilateral testicular weight (RBTW). They found that the TIR group had a lower RBTW than the therapy group 4 weeks after starting reperfusion. Kazaz et al[7] randomly divided 18 Sprague-Dawley rats into three groups. In the T/D group rats, the left testis was rotated 720° for 4 h followed by detorsion for 2 h. The results showed that the histopathological score, seminiferous tubule diameter, and germinal-epithelium thickness were significantly lower in the T/D group than in the control group. Meanwhile, degeneration and cell loss in the seminiferous-tubule epithelium were observed in the T/D group. Similarly, Chi et al[11] rotated the testis 720° for 90 min followed by detorsion for 60 min. They found that TIR injury exerted histopathological effects, such as sloughing, decreased cellularity, hemorrhage, and necrosis. Many previous studies[2,25-27] have revealed that TIR induces histological alterations in the tunica albuginea, typical interstitial space injuries such as edema, dilated and congested blood vessels, necrotic seminiferous tubules, disintegrated interstitial tissue, atrophic seminiferous tubules, spermatogenetic-cell accumulation in the tubular lumen, and occasional irregularities in the seminiferous-tubule epithelium.

Spermatogenesis is an important process affected by testicular torsion. TIR causes DNA damage, protein-synthesis inhibition, and spermatogonia arrest, all of which impair sperm production[3]. Sperm membranes contain high levels of unsaturated fatty acids and are extremely sensitive to oxidative stress[28]. This process results in germ-cell-specific apoptosis and infertility[29]. Defective sperm function, the most prevalent cause of male infertility, is difficult to treat[30]. TIR injury upon testicular torsion disrupts spermatogenesis, and its damage can be permanent with short-term suppression of testosterone secretion[31,32]. This phenomenon may make the testes aspermatogenic[33]. Similarly, Ikebuaso et al[34] indicated that TIR could reduce testosterone levels, sperm count, and sperm motility. Reduced sperm motility could be attributed to ROS-induced damage to proteins, specifically electron-transport-chain proteins, lipids, and DNA, and consequent impairment of mitochondrial function and ATP production[35]. Koksal et al[30] reported that TIR could significantly increase the rate of abnormal sperm, indicating testicular damage, as demonstrated by histological analysis. The serum level of testosterone was lower in the TTD group than in the other groups, which may be related to germ-cell apoptosis and injury to Leydig cells of testicular tissue[12,36]. Other studies[32,37] have also reported that testicular injury decreases serum testosterone level. Luteinizing hormone (LH), testosterone, and follicle stimulating hormone (FSH) play an important role in controlling testicular functions. LH released by the pituitary gland acts upon the Leydig cells to produce testosterone. FSH participates in spermatogenesis initiation and germ-cell maturation. A previous study[38] showed that the serum levels of LH, FSH, and testosterone were significantly reduced in I/R rats compared with the control group, suggesting that I/R induced loss of hormonal activities.

Oxidative Stress Plays Important Roles in TIR Damage

Testicular torsion and detorsion induce morphological and biochemical changes by both ischemia and reperfusion of the tissues[7]. As the primary pathophysiological consequence of testicular torsion, TIR injury always leads to ROS

overproduction, thereby impairing seminiferous epithelium and finally giving rise to male infertility or subfertility[2]. Oxidative stress-related damage during the reperfusion period is more critical than ischemia-induced damage[39]. Oxidative stress characterized by an imbalance between ROS and antioxidative-defense systems is the main cause of testicular reperfusion injury[24]. Several studies[36,40] have shown that TTD increases oxidative stress and reduces the activity of antioxidant enzymes. Evidence[41] suggests that ROS have important functions in the pathogenesis of TIR injury, but the exact mechanism is unclear. ROS react with unsaturated fatty acids in cell membranes. Excessive ROS generation damages tissue by oxidizing cell-membrane lipids, proteins, and DNA[2,20,42]. It also regulates many genes whose expression affects cell-cycle regulation, cell proliferation, and apoptosis[43,44].

Calcium influx into neutrophils during ischemia increases cellular NADPH oxidase (NOX) activity, triggering the release of free radicals during reperfusion[45]. NOX is a major endogenous source of cellular ROS, and an increase in its activity is a possible trigger of ROS-mediated death signals in spermatozoa[46]. NOX5 is expressed in spermatocytes, and it plays an essential role in germ-cell proliferation and fertilization[47]. NOX-derived ROS contribute to spermatocyte maturation and acrosome formation and capacitation through NOX-dependent apoptosis[48]. In mouse spermatogonial stem cells (SSCs) with NOX1 knockdown, ROS is depleted, and SSCs show reduced self-renewal division through activation of the p38 mitogen-activated protein kinase (MAPK) and Jun N-terminal kinase (JNK) pathways[49]. Later, the same study[50] confirmed that NOX3 is another ROS contributor to SSC self-renewal by using short hairpin RNA. ROS generated by NOX4 in male germ cells serve as a second messenger in signaling pathways and regulate gene expression[51]. Apocynin, a plant-derived medicinal herb that interferes with the assembly of the functional NOX complex, can prevent I/R-induced testicular damage of spermatogenesis by inhibiting oxidative stress[46].

Biochemical markers of oxidative stress are more sensitive indicators of tissue damage and can be detected much earlier than histological changes[52]. ROS, except hydrogen peroxide, are difficult to measure directly because of their high reactivity and short life-span[53]. MDA is produced after ROS-induced lipid peroxidation in the cell membrane, and it is extensively used as a sensitive biomarker of ROS[5]. Many physiological defense systems fight against oxidative stress[54]. Afolabi et al[35] reported that oxidative stress can induce testicular reperfusion injury, as evidenced by the increased production of hydrogen peroxide, nitrite, and MDA. Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) protect tissues from ROS effects. Under normal conditions, ROS and their effects are eliminated by the endogenous antioxidant system[24]. Shokoohi et al[44] reported that testicular TTD decreases SOD and GPx levels. Meanwhile, some drugs protect testicular tissues against TIR by inhibiting free-radical generation and increasing antioxidant defense. Antioxidant agents such as zinc, melatonin, polydatin, and apocynin reportedly exert protective effects by decreasing MDA and ROS levels and increasing SOD, GPx, and CAT levels[55,56].

Nuclear factor-erythroid 2-related factor 2 (Nrf2) is a key antioxidant response element controlling the activation of antioxidant enzymes[57]. It can be released in a Keap-1-dependent or Keap-1-independent manner in the presence of environmental insults[58]. The released Nrf2 then translocates into the nucleus and binds to antioxidant response elements, leading to the upregulation of downstream II phase enzymes, such as heme oxygenase-1 (HO-1) and NADPH quinone oxidoreductase-1 (NQO-1)[15]. HO-1 increases host antioxidant defenses, suggesting that it exerts a protective effect on testicular tissue after TTD[59]. NQO-1 decreases endogenous quinones such as coenzyme Q10 to generate stable hydroquinones with excellent antioxidant properties. Mohamed et al[60] showed that the protein expression of the antioxidant Nrf2 in I/R rats significantly decreased in extent and intensity. This result is consistent with a previous study[61] linking the absence of Nrf2 to the disruption of sperm viability, motility, and count owing to testicular oxidative damage. A previous study[62] showed that the testicular expression of nuclear Nrf2 significantly decreased in the I/R group, whereas the expression levels of HO-1 and NQO1 were sharply downregulated. The abovementioned studies[15,57-62] suggest a critical role for Nrf2 in preventing the oxidative disruption of I/R-induced testicular damage.

Excessive ROS generation into the environment during reperfusion induces oxidative stress in the testicular parenchyma, damages the cell genome, and induces apoptosis by activating caspase cascades. All of these events are directly related to increased necrosis in testicular tissue[27]. Activated

cytochrome C converts pro-caspase-3 to active caspase-3, which induces testicular cell apoptosis through nuclear destruction, DNA fragmentation, protein and cytoskeleton degradation, and phagocytosis. Oxidative stress phosphorylates the Bcl-2 protein family, tilting the balance between pro- and anti-apoptotic proteins in favor of the former, such as the Bax protein. I/R injury increased the number of TUNEL-positive cells in the seminiferous tubules (including spermatogonia, primary spermatocytes, and spermatids), upregulated the expression of pro-apoptotic genes, such as Bax and caspase-3, and downregulated the expression of Bcl-2[63]. As mentioned above, ROS-induced testicular cell apoptosis participates in testicular I/R damage. The relationship between I/R-induced oxidative stress and the onset of testicular damage is shown in Figure 1.

Drugs Treatment in TIR Damage

To date, treatment for TIR injury in the clinical setting is unavailable. Given the pathophysiology of TIR, antioxidant treatment could prevent I/R injury. Therefore, various antioxidant drugs, enzymes, and chemical agents could be used to increase the activities of antioxidant enzymes, inhibit oxidative stress, and prevent I/R injury[27]. Sildenafil citrate[18], N-acetylcysteine[64], quercetin[65], and melatonin[66] have been used to decrease ROS levels in experimental studies[18,64-66] of TIR. Ghasemnejad-Berenji et al[67] showed that metformin pretreatment reduces MDA and caspase-3 levels and normalized antioxidant-enzyme activities 4 h after detorsion. Germ-cell apoptosis was also significantly decreased, and MSTD and sperm functions significantly improved. Yazdani et al[68] demonstrated that pre- and post-reperfusion nortriptyline reduced MDA and caspase-3 levels and normalized antioxidant-enzyme activities in a dose-dependent manner. Germ-cell apoptosis significantly decreased, and MSTD and sperm functions significantly improved. Inhibition of the mitochondrial-permeability transition pore was probably involved in the protective effects of nortriptyline against testicular T/D cell damage. Jafari et al[69] showed that topiramate administration significantly increased GSH levels and GPx, CAT, and SOD activities and decreased

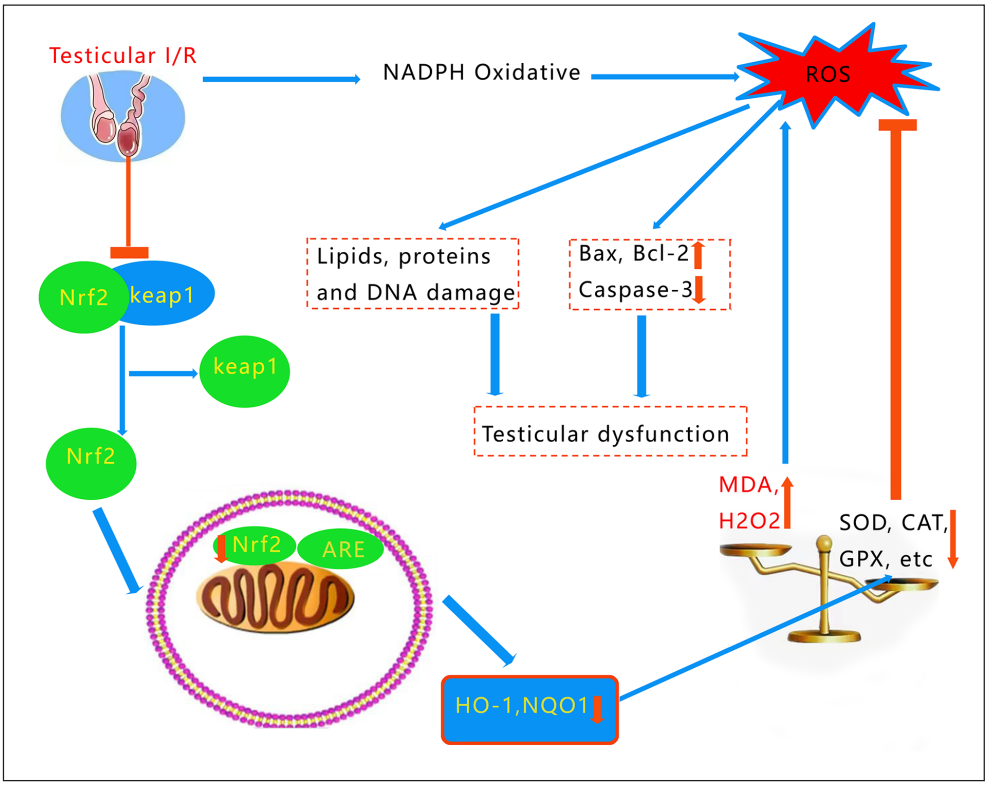

Figure 1. Scheme summarizing the relationship between testicular I/R-induced oxidative stress and the onset of testicular dysfunction. MDA: malondialdehyde; ROS: reactive oxygen species; SOD: superoxide dismutase; CAT: catalase; GPX: glutathione peroxidase; Nrf2: nuclear factor-erythroid 2-related factor 2; ARE: antioxidant response element. HO-1: heme oxygenase-1; NQO-1: NADPH quinone oxidoreductase-1

Table I. Literature review of various drugs/plants/herbs showing modulatory effects on testicular ischemia/reperfusion injury.

Drugs/plants	Dose/mode/duration	Effects observed on reproductive functions	References
α-Lipoic Acid	100 mg/kg α-lipoic acid was administered i.p. 30 minutes prior to detorsion.	α-lipoic acid pretreatment reduced testicular cell damage and decreased TUNEL and caspase-3-positive cells. Additionally, α-lipoic acid administration decreased the GPx and SOD activity and increased the MDA levels.	70
Apocynin	20 mg/kg apocynin was administered i.p. at minute 210 of ischemia.	Apocynin significantly decreased MDA, TOC and the OSI, and significantly increased SOD and CAT level. There was a significantly increase in the number of giant, degenerated and desquamated cells in the I/R group. Apocynin significantly improved these histological alterations.	56
Berberine (BBR)	200 mg/kg BBR was given i.p. 30 min before detorsion.	In T/D group, tissue MDA, TOS, and OSI levels were higher than control group. These increases were significantly reversed with BBR pretreatment. BBR pretreatment recovered the Johnsen scores.	7
Baicalin	25, 50 and 100 mg/kg baicalin was administered i.p. Each dose was given twice, the first 30 min before and the second 12 h after testicular detorsion.	Baicalin, in a dose-dependent manner, decreased the T/D-induced elevations of testicular MDA, NO, TNF-a, Bax, cytosolic cytochrome c and caspase-3 and caspase-9 activities. Baicalin, dose-dependently, attenuated the reductions of Bcl-2, and GPx and SOD activities in testicular tissues resulted from T/D. In addition, baicalin ameliorated the histopathological testicular tissue damage and reduced the expression of Fas ligand in rat testes exposed to torsion/detorsion in a dose-dependent manner.	4
Crocin	50 and 100 mg/kg crocin was administered i.p. 30 min before torsion period.	Crocin could significantly improve the histopathological parameters in both treatment groups compared to the T/D group. T/D reduced SOD and GPx activity and testosterone level significantly (except for GPx) compared to the sham group. However, crocin administration could significantly reverse them. Additionally, crocin reduced the amount of MDA significantly in the high-dose treatment group in comparison to T/D group.	71
Carvacrol	73 mg/kg carvacrol was administered i.p. half an hour before detorsion.	Apoptotic cells and serum MDA levels were significantly decreased and Kir6.2 activation was significantly increased in Carvacrol-administered I/R group.	12
Cysteamine	100 or 200 mg/kg cysteamine through i.p. route for one week before T/D-induced testicular reperfusion injury.	Significant increase in H2O2, MDA and nitrite but reduction in SOD, GPx, GSH, GST and total thiol in the testicular tissue of IRI rats was reversed by cysteamine. Serum MPO and TNF-α were significantly elevated in IRI, while treated IRI rats showed decrease in tissue level of the inflammation markers. Reduced sperm motility in IRI was significantly reversed by cysteamine. Increased tissue expression of bax and caspase-3 was reversed by cysteamine.	35
Coenzyme Q10	10 mg/kg CoQ10 was injected i.p. 30 minutes before detorsion.	CoQ10 administration before the reperfusion period of testicular torsion provides a significant decrease in testicular lipid peroxidation products and expressions of inducible NOS, eNOS, and germ cell-specific apoptosis.	8

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Table I *(Continued)*. Literature review of various drugs/plants/herbs showing modulatory effects on testicular ischemia/reperfusion injury.

Drugs/plants	Dose/mode/duration	Effects observed on reproductive functions	References
Carvedilol (CVD)	2 mg/kg CVD was administered i.p. 30 min before detorsion.	Activities of SOD and GPx in serum and testicular tissues were increased by I/R, but administration of CVD decreased these levels. Significantly increased MDA levels in serum and testicular tissues were decreased by CVD treatment.	72
Cordycepin	10 mg/kg cordycepin was administered i.p. 15 min before detorsion.	Cordycepin groups were compared with the other groups, and there was a statistically significant decrease in TNF-α and MDA levels. Increased TAS levels were observed in the cordycepin group compared with the control group. TOS levels were significantly increased in the I/R groups but decreased in the cordycepin group. Histopathological evaluations revealed that the spermatozoa count was decreased in the I/R groups. However, there was an increase in the cordycepin group, as well as a statistically significant difference between the IR and cordycepin groups. Finally, edema and inflammation were increased in the IR groups, but decreased in the cordycepin group.	73
Colchicine	1 mg/kg solution of colchicine was given p.o. 30 minutes before detorsion (I/Rc1); continued p.o. once daily for five days (I/Rc5).	Decrease in MDA protein levels and increase in SOD, CAT and GPx levels achieved in I/Rc5 group when compared to I/R group. MSTD, GECT, and JS were better in I/Rc5 than I/R, which showed the natural course of I/R damage in testis. Caspase 3 positivity, as an apoptosis indicator, were significantly lower in I/Rc5 group than other groups.	74
Diacerein (DIA)	50 mg/kg DIA was administered i.m. to rats in the presence or absence of TIR.	DIA was able to normalize both testicular weight, serum testosterone and cholesterol levels with attenuation of oxidative stress parameters along with amelioration of histopathological changes and IL-1β immuno-staining induced by TIR.	37
Dexmedetomidine (Dex)	50 or 100 µg/kg Dex i.p. at minute 180 of ischemia and then detorsion	Increasing doses of Dex significantly increased TAS, and significantly decreased OSI. Dex 100 µg/kg statistically significantly increased the tissue TAS and OSI when compared with Dex 50 µg/kg but authors did not find significant change in the tissue TOS.	75
Dopamine and vitamin C	Dopamine 0.01 mg/kg or/and vitamin C 100 mg/kg just before TD.	Testicular torsion caused a significant decrease in the percentage of spermatogenesis and seminiferous tubules diameters compared with the control and sham groups. Administration of dopamine, vitamin C and their combination increased abovementioned parameters and decreased serum MDA levels significantly. However, vitamin C had better results than the other treatments.	76
Etoricoxib	50 and 100 mg/kg etoricoxib was adminstrated orally before detorsion.	The levels of MDA, MPO, IL-1β and TNF-α were significantly higher, and the levels of total glutathione and glutathione reductase were significantly lower in the TTD group, compared to the ETD-50 and ETD-100 groups.	77
Edaravone	1 or 10 mg/kg edaravone was adminstrated i.p. before ischemia.	The levels of NO2 -NO3, MDA, 8-Hydroxide oxyguanosine, MPO and HSP 70 and its mRNA, and histological variables, were significantly greater in the I/R group than in the control, and these variables were ameliorated by treatment with edaravone.	78

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Table I *(Continued)*. Literature review of various drugs/plants/herbs showing modulatory effects on testicular ischemia/reperfusion injury.

Drugs/plants	Dose/mode/duration	Effects observed on reproductive functions	References
(−)-epigallocatechin gallate (EGCG)	150 mg/kg EGCG was orally administered 1 hour before reperfusion.	Serum SOD activity was significantly higher in EGCG group than in I/R group. The ratios of bilateral testicular weight, MSTD, and germinal epithelial cell thickness were significantly higher in EGCG group than in I/R group.	24
Glutathione	25 mg/kg gluthatione was injected i.v. before performing detorsion; onset time of agent is 15 min.	The lowest MDA expression was observed in the treated group. Grading of the histopathological damage to seminiferous tubules showed the damage to be worst in T/D and least in treated group.	79
Grape seed extract (GSE)	100 mg/kg GSE was administered by oral gavage over seven days before torsion.	Prevented the rise in MDA, apoptosis and eNOS expression and improved testicular morphology and Johnsen's score in GSE treated group.	80
Ginkgo biloba (EGb 761)	50 mg/kg EGb 761 was administered orally only once, 40 min prior to detorsion.	EGb 761 treated animals showed an improved histological appearance in I/R group. Significant reduction in the activity of TUNEL and eNOS in testes tissue of I/R treated with EGb 761 therapy. Electron microscopy of the testes of rats demonstrated that EGb761 pretreatment was particularly effective in preventing the mitochondrial degeneration, dilatation of sER and enlargement of intercellular spaces in both Sertoli and spermatid cells in I/R treated animals.	81
Ganoderma lucidum	20 mg/kg ganoderma lucidum per day via gastric gavage for 7 days.	G. lucidum treatment was found to have prevented the T/D-induced I/R injury by decreasing MDA levels of the testis. SOD, CAT and GSH activities were decreased in I/R group, while they were increased and significant improvement in the tube diameter was observed G. lucidum group.	82
Hesperidin	50 mg/kg hesperidin was administered i.p. 30 min before detorsion.	In the T/D group, testicular MDA levels were increased significantly whereas SOD, CAT and GSH levels were decreased compared to the control and other groups. However, hesperidin ameliorated the effect of T/D and biochemical values became closer to normal. In addition, the histological examinations showed that T/D caused damage in the testis, but hesperidin reduced this effect.	83
Hydroalcoholic extract of Fumaria parviflora (FP)	250 mg/kg FP was administered orally for 14 days before detorsion.	The Johnson's score, MSTD and height (thickness) of seminiferous tubule epithelium were significantly increased in TDFP as compared to TD group. The gene expression of Bcl-2, level of serum testosterone hormone and antioxidant parameters – GPx and SOD – were significantly higher in TDFP group than TD group. The index of apoptosis, the gene expression of Bax and the level of MDA were significantly higher in TD group than in TDFP groups.	44
Korean red ginseng (KRG)	100 mg/kg KRG was administered orally for 4 weeks before detorsion.	Testicular weight was significantly higher in the T+K group than in the T group. The mean level of ROS and SOD production was significantly lower in the KRG group. Upon histologic evaluation, the KRG group had a germinal epithelial layer that appeared nearly normal.	84

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Table I *(Continued)*. Literature review of various drugs/plants/herbs showing modulatory effects on testicular ischemia/reperfusion injury.

Drugs/plants	Dose/mode/duration	Effects observed on reproductive functions	References
Lipoxin A4 (LXA4)	0.1, 1.0, and 10 mg/kg LXA4 was adminstrated i.p. in single dose 1 h before detorsion.	GPx and SOD levels were significantly increased, and MDA levels significantly reduced in LXA4-pretreated groups compared to T/D. LXA4 also reverted IL-2 and TNF-alpha to basal levels and improved the expression of IL-4 and IL-10 in LXA4-pretreated groups. Moreover, the expression of NF-kB was downregulated in LXA4-pretreated groups. LXA4 treatment also showed an improved testicular morphology and decreased apoptosis in testes.	85
Liraglutide	0.6 mg/kg liraglutide was adminstrated i.p. before detorsion.	In the reperfusion group, CAT and SOD values were increased, NO and MDA values were decreased after administration of liraglutide. In addition, GPx values were significantly increased in I/R + liraglutide administered group compared to reperfusion group. Apaf-1 and iNOS activity were significantly decreased with the addition of liraglutide treatment to the I/R group.	86
Myricetin	1 mg/kg myricetin l was given i.p. 30 min before detorsion.	There was a statistically significant decrease in MDA values in myricetin group compared to TD group. There was no significant difference in the statistical analysis of SOD and CAT values.	9
Milrinone	0.5 mg/kg of milrinone was administered i.p. immediately after testicular torsion.	Histopathological examinations indicated a dramatic improvement in terms of inflammation, hemorrhage, edema, congestion, Cosentino and Johnson scores in treated group compared to TD group. SOD, GSH-px activity and TAS levels increased significantly in treated group compared to TD group. MDA, protein carbonyl, IL- 1β, TNF-α and TOS levels decreased in treated group compared to TD group. Tissue biochemical analyses demonstrated an increase in SOD and GSH-px activity in treated group compared to TD group, while PC and MDA levels were reduced.	87
Minocycline	160 mg/kg minocycline was adminstrated orally 30 min before detorsion and then continued for 8 weeks.	Johnson's score, the height of seminiferous tubule epithelium, the MSTD, SOD, GPx and CAT, were significantly enhanced in the I/R + minocycline group compared with the I/R group. The administration of minocycline led to a marked decrease in expression levels of Caspase-3, Bax, IL-1β and TNF-α genes, and a remarkable increase in expression levels of Bcl-2, 3β-HSD and 17β-HSD3 genes compared with the I/R group.	63
Memantine	10 mg/kg memantine was administered i.p. 30 min before detorsion.	The testicular MDA values in the T/D+memantine group were significantly lower than those in the T/D group. Additionally, significant decreases occurred in CAT and SOD activities in the T/D group compared with sham operated group. These values were significantly greater in the memantine group than in the T/D group. Furthermore, after induction of T/D, histopathological evaluations also revealed severe testicular damages which were improved by memantine administration.	27
Melatonin	10 mg/kg melatonin was administered i.p. daily beginning 15 minutes before detorsion and continued for the following 7 days.	Melatonin prevented the rise in MDA and total NO levels and improved Johnsen score, tissue and plasma inhibin B, and tissue glutathione levels, along with a decrease in plasma RSH level.	14

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Table I ***(Continued).*** Literature review of various drugs/plants/herbs showing modulatory effects on testicular ischemia/reperfusion injury.

Drugs/plants	Dose/mode/duration	Effects observed on reproductive functions	References
Mannitol	1 mg/kg mannitol was administered i.v. just after testicular torsion.	Testicular tissue structure was significantly better in the mannitol-treated group, demonstrating a protective effect. Similar findings were also shown for the PCNA index and antioxidant activity; both were higher in the mannitol group than in the no-treatment or saline groups. The apoptotic index was also significantly lower in the mannitol-treated group compared with the no-treatment or saline groups.	88
Montelukast	10 mg/kg montelukast was administered i.p. 30 minutes prior to and during detorsion.	MDA levels and MPO activity were found to be elevated in the I/R groups and accompanied by a significant decrease in glutathione levels when compared to the sham groups. I/R significantly increased iNOS activity and germ cell apoptosis compared to the sham groups. Montelukast treatment significantly reversed all of these parameters and achieved comparable results with the sham groups.	89
Matricaria chamomilla Extract (MC)	300 mg/kg MC was administered i.p. 30 minutes before detorsion.	The levels of SOD, GPx, and testosterone hormone were significantly decreased in T/D group as compared to sham group, while these parameters increased in MC group as compared to T/D group. During ischemia, the MDA levels increased; however, treatment with MC extract decreased the MDA levels.	90
Metformin	300 mg/kg metformin was administered i.p. and surgical procedure was performed immediately.	Metformin pretreatment reduced MDA and caspase-3 levels and normalized antioxidant enzyme activities 4 hr after detorsion, and germ cell apoptosis was significantly decreased, and the MSTD, as well as sperm functions, was significantly improved.	67
Nortriptyline	2, 10 and 20 mg/kg nortriptyline was administered i.p. 30 and 90 min after torsion.	Pre-and post-reperfusion nortriptyline could reduce MDA and caspase-3 levels and normalize antioxidant enzyme activities dose-dependently. Germ cell apoptosis was significantly decreased, and the MSTD, as well as sperm functions, were significantly improved. Inhibition of mitochondrial permeability transition pore is probably involved in protective effects of nortriptyline against testicular T/D cell damages.	68
N-acetylcysteine (NAC)	20 mg/kg NAC was given i.p. 30 minutes before detorsion and following 5 days after detorsion.	GPx activities were increased in the T and T+NAC groups compared with the control. Seminiferous tubule diameter thickness is decreased in the torsion group compared with the control group and decreased in the T+NAC group compared with the torsion group.	64
Nimesulide (NIM)	50 mg/kg and 100 mg/kg NIM was administered i.p. 2 h before I/R procedures.	tGSH and COX-1 levels were increased in the NIM-50 and NIM-100 groups compared to IR group. The levels of COX-2, MDA and TNF-α were lower in the NIM-50 and NIM-100 groups than in the IR group.	26
Nitroglycerin (NG)	5 mg/kg NG was administered i.p. immediate after detorsion.	Testicular T/D significantly reduced sperm viability, motility, and normal morphology, whereas the NG administration markedly increased the percentage of live, motile, and normal spermatozoa. The NG-treated group exhibited significantly reduced MDA concentrations as well as elevated levels of GPx and CAT compared to the T/D group. Induction of testicular torsion significantly reduced Johnson's score, GESCT, and MSTD, and markedly increased the Cosentino's score, while NG injection significantly increased Johnson's score, GESCT, and MSTD and reduced the Cosentino's score.	91

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Table I *(Continued)*. Literature review of various drugs/plants/herbs showing modulatory effects on testicular ischemia/reperfusion injury.

Drugs/plants	Dose/mode/duration	Effects observed on reproductive functions	References
Proxeed plus	1,000 or 5,000 mg/kg proxeed plus was administered before inducing reperfusion.	The prophylactic treatment of the I/R injured rats with PP at various doses resulted in significant increases in the serum and tissue antioxidative defense capacities (SOD, reduced glutathione, CAT, glutathione-s-transferase, and GPX), sex hormones (luteinizing hormone, follicle-stimulating hormone, and testosterone); PP also reduced MDA and hydrogen peroxide, serum iNOS and apoptotic parameters (Caspase-3 and Caspase-9) in comparison to the results in the I/R untreated rats. PP ameliorated histological changes of I/R injured rats, i.e., increased spermatogenetic activity, seminiferous tubular diameter, Leydig cell mass, and reduced expressions of testicular induciblen iNOS.	38
Pirfenidone	325 mg/kg pirfenidone administered via oral gavage immediately after ischemia.	Pirfenidone treated group showed increased SOD and GSH-Px activities compared with TD group.	92
Picroside II	10 mg/kg picroside II was administered i.p. 30 minutes before testis detorsion.	The seminiferous tubules were damaged in I/R rats, but picroside II alleviated the changes induced by I/R. The increased level of apoptosis was reversed by picroside II. The activities of HO-1, MPO, NOX, XO and MDA content were increased, and the SOD activity was decreased in I/R and could be reversed by picroside II. Western blot showed that the expression of iNOS, nNOS and eNOS were increased in I/R; however, they were decreased after picroside II treatment.	93
Psoralea corylifolia	1.0 g/kg/day Psoralea corylifolia was adminstrated orally after detorsion for 3 months.	Psoralea corylifolia treatment significantly decreased MDA level and significantly increased CREM expression and spermatogenesis in ipsilateral testes, compared with T/D group.	43
Phoenix dactylifera (DP)	500 mg/kg DP was adminstrated orally after detorsion for 10 days.	DP-treated group showed significantly decreased serum MDA, TOS, and OSI levels, but increased TAS levels. Ipsilateral-twisted testicular tissue in the DP-treated group showed moderate-to-mild changes. Contralateral testicular tissue in the T/D group had a mild-to-moderate tissue injury; meanwhile, treated group revealed normal-to-mild changes. Spermatogenesis was significantly improved in DP-treated group when compared with the T/D group.	94
Paeonol	50 or 100 mg/kg was administered by an intragastric tube for three consecutive days before testicular I/R injury.	Pretreatment with paeonol prevented the drop in serum testosterone, alongside with improvement of testicular malondialdehyde and GSH levels plus SOD activity. Paeonol restored the normal spermatogenesis and prevented I/R-induced increase in TNF-α, HIF-1α and HSP70 gene expression in addition to IL-1β and IL-6 immunostaining and reduction in Nrf2 protein expression.	60
Plantago major leaf extracts (PM)	50 or 100 mg/kg i.p. for seven days after detorsion.	Decreased MDA level and increased CAT level in two dose of PM treated group than I/R group. PM also prevented I/R-induced cell damage and histological changes in the testicular tissue.	3
Pentoxifylline (PTX)	40 mg/kg PTX was administered i.p. 30 min before detorsion.	Significant adverse changes were observed in the TD group for histological variables, sperm count, oxidative marker, testosterone hormone, Bax, Bcl-2 and caspase-3 expression. The parameters studied in the group receiving PTX improved in comparison with the TD group.	32

Continued

Table I ***(Continued).*** Literature review of various drugs/plants/herbs showing modulatory effects on testicular ischemia/reperfusion injury.

Drugs/plants	Dose/mode/duration	Effects observed on reproductive functions	References
Polydatin (PD)	20 mg/kg PD was administered i.p. 30 min before detorsion.	Compared with the T/D group, PD pretreatment significantly ameliorated the morphological damage, lowered the Cosentino histological score and increased the mean number of germ cell layers and Johnsen's testicular biopsy score. In addition, PD treatment markedly decreased MDA levels and upregulated CAT, GPx and SOD activities. Furthermore, PD decreased T/D-induced germ cell-specific apoptosis, attenuated the activation of caspase-3, caspase-8, caspase-9 and poly(ADP-ribose) polymerase and increased the Bcl-2/Bax ratio.	55
Pomegranate (Punica granatum) juice (PJ)	The rats were given 0.4 ml/day PJ orally over a period of eight weeks prior to surgery.	PJ treatment significantly decreased the SOD and MDA levels in both the serum and testicular tissue of the rats. PJ treatment significantly improved the concentrations of spermatids, spermatocytes and spermatogonia compared with those in the I/R group.	28
Probucol	300 mg/kg of probucol was administered i.p. to each rat at reperfusion.	The probucol-treated group showed significant decreases in E-selectin protein expression, MPO activity, and MDA level and significant increase in testicular spermatogenesis in the ipsilateral testes, compared with the I/R group.	95
Pterostilbene	Pterostilbene (50 mg/kg) was simultaneously injected i.p.	Germ cell apoptosis and MDA level significantly increased whereas TAC significantly decreased in IR group; moreover, abnormal morphology and impaired spermatogenesis were observed in IR group. In contrast, treatment with pterostilbene inhibited lipid peroxidation and apoptosis induced by ROS and restored the antioxidant capacity.	96
Quercetin (QE)	QE (20 mg/kg) was injected i.p. or injected epididymally (IE) after 60 minutes of torsion.	QE administration significantly decreased MDA and TOS, increased GPx and TAS, histopathological damage, and germinal cell apoptosis compared with the TD group. Most importantly, no significant differences in the biochemical parameters, histopathological changes, and germinal cell apoptosis between the IP-QE and IE-QE groups were found.	11
Rutin	30 mg/kg rutin was administered intravenously at the time of detorsion.	The rats treated with rutin had a significant decrease in MDA level and had significant increases in SOD, CAT activities, and spermatogenesis in ipsilateral testes, compared with torsion-detorsion group.	42
Resveratrol (RSV)	RSV (20 mg/kg) was injected i.p. at 60 min of torsion.	RSV significantly lowered MDA, NO, and TOS levels and TAS consumption. RSV (mean grade 3.00) had lower testicular injury grades than model group (mean grade 3.45).	65
Rosiglitazone	Rosiglitazone (4 mg/kg) was injected 30 min before detorsion.	Rosiglitazone group had better testicular architecture. Similar findings were also shown for lipid peroxidation by evaluating the MDA activity. The levels of inflammation as evaluated by the MPO activity, the levels of TNF-α, IL-1 and IL-6 and the expressions of ICAM-1 were prominently suppressed in Rosiglitazone group as well.	97

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Table I *(Continued)*. Literature review of various drugs/plants/herbs showing modulatory effects on testicular ischemia/reperfusion injury.

Drugs/plants	Dose/mode/duration	Effects observed on reproductive functions	References
Simvastatin	Simvastatin (1 or 5 mg/kg) was administered immediately after detorsion or immediately after sham operation.	Histological findings revealed severe injury in testes of the T/D and simvastatin (1 mg/kg) groups while testes in the simvastatin (5 mg/kg) group showed moderate injury. MPO activity, cytokines, NO and MDA in testes in the simvastatin (5 mg/kg) group were significantly lower than those in the T/D group. Testicular concentrations of NF-kB in nuclear extracts and phosphorylated inhibitor-B in cytosolic extracts in the simvastatin (5 mg/kg) group were significantly lower than in the T/D group.	98
Stevia rebaudiana aqueous extract	500 or 1,000 mg/kg S. rebaudiana extract i.p. 30 min before detorsion.	Testicular tissues of both treatment groups revealed reduced histopathological alterations. Significantly higher MDA level was observed in T/D group than sham or no-treatment groups. Compared with torsion/detorsion group, S. rebaudiana extract significantly reduced MDA level in treatment groups. T/D group had significantly lower GPX and SOD activities than sham or no-treatment groups, and these parameters showed significant increase in treatment groups compared with T/D group.	99
Sinapic acid (SA)	10 mg/kg and 20 mg/kg, SA was administered by single i.p. injection, 30 min before reperfusion.	SA significantly reversed testicular damage, oxidative stress, inflammation and cell death induced by reperfusion and restored antioxidant enzyme activities suppressed by reperfusion.	100
Salvia miltiorrhiza (SM)	200 mg/kg SM was administered i.p. 30 minutes before detorsion.	Testicular I/R significantly reduced sperm motility, viability, and normal morphology, while SM extract administration markedly increased sperm motility, and normal morphology. Induction of testicular T/D caused a significant increase in the level of MDA and notable decline in the levels of GPX, CAT, and TAC both in plasma and testis tissue, whereas administration of SM extract significantly decreased MDA level and increased GPX, CAT, and TAC levels in plasma and testicular tissue. Histopathological parameters including MSTD, GECT, MTBS, and TCT were significantly lower in the T-D group, while pretreatment with SM extract markedly increased MSTD, GECT, and MTBS amounts.	13
Salvianolic acid B	10 mg/kg salvianolic acid B was administered i.p. after detorsion.	In the salvianolic acid B-treated group, XO protein expression and MDA concentration in ipsilateral testes decreased significantly, while spermatogenesis increased significantly, compared with the testicular ischemia-reperfusion group.	5
Sesamol	Sesamol treatment at the dose of 50 mg/kg through the tail vein at reperfusion.	Sesamol treatment resulted in a significant reduction in the MDA level and significant increase in CREMτ expression and spermatogenesis in ipsilateral testis.	101
Sumatriptan	0.1, 0.3 and 1 mg/kg sumatriptan was adminstrated i.p. after the induction of testicular torsion.	After inducing testicular T/D, SOD activity was decreased, whereas administration of sumatriptan significantly increased SOD activity in bilateral testes. After induction of T/D, macroscopic and histological analyses also showed severe damages which were improved by sumatriptan injection.	102

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Table I *(Continued).* Literature review of various drugs/plants/herbs showing modulatory effects on testicular ischemia/reperfusion injury.

Drugs/plants	Dose/mode/duration	Effects observed on reproductive functions	References
Selenium	Ten minutes before detorsion, an i.p. injection of 0.5 mg/kg selenium for 7 days.	SOD levels of the selenium groups were higher than those of the I/R group. Furthermore, MDA levels of the I/R group were higher than those in the other three groups. TAC levels were lower in the I/R group than the selenium group. GSH levels of the I/R group were significantly lower than those in the selenium group.	103
Sildenafil citrate (SC)	0.7 or 1.4 mg/kg of SC was adminstrated i.p. 1 h before detorsion.	Administration of low-dose SC prevented the increases in MDA and NO levels and decreases in GPx activities and GSH values induced by testicular torsion. However, administration of high-dose SC had no effect on these testicular parameters. Reversal of histopathological changes were observed in SC groups.	18
Topiramate	100 mg/kg topiramate was injected intraperitoneally 30 min before detorsion.	Administration of topiramate significantly increased GSH level and GPx, CAT and SOD activities and decreased MDA level in testis tissue as compared to T/D group.	69
Thymoquinone (TQ)	TQ (50 mg/kg) was administered i.p. before the 30 min ischemic period.	The SOD activity and MDA levels in the torsion group were significantly higher than those of the sham group. Thymoquinone administration significantly reduced these levels. Torsion significantly increased active-Caspase3 and Bax expression, which was decreased by thymoquinone. However, thymoquinone significantly reduced the apoptotic index.	104
Urapidil	Urapidil 0.5 mg/kg was administered i.p. 30 min before detorsion.	The rats treated with urapidil had a significant decrease in the MDA level and apoptosis and significant increases in the SOD and GPx activities in ipsilateral testes compared to the T/D group.	105
Zinc	5 mg/kg zinc i.p. for 3 weeks.	The highest erythrocyte and testis GSH values were found in zinc group. However, zinc-supplemented group had higher inhibin-B and spermatogenetic activity.	66

MDA levels in testis tissue compared with the T/D group. The present review summarizes these plants/drugs with protective effects against TIR, and they are listed in Table I. However, none of these agents have been used as adjunctive therapy in torsion repair in humans. Accordingly, randomized clinical trials are needed before the clinical use of thse plants/drugs.

Conclusions

Despite the unequivocal benefit of blood reperfusion to ischemic tissue by detorsion, reperfusion can elicit a cascade of adverse reactions that elicit paradoxical insults even with successful surgical repair. In animal models of TTD, various medicinal plants/drugs have been successfully used as oxygen-radical scavengers to reduce TIR injury. However, all of the above drugs for TTD are only at the experimental level, and their widespread clinical application is limited by a lack of clinical trials.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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