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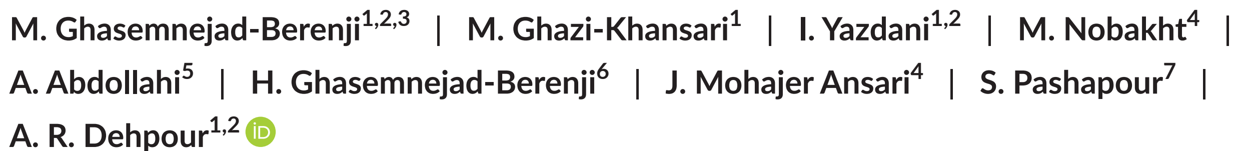
Effect of metformin on germ cell-specific apoptosis, oxidative stress and epididymal sperm quality after testicular torsion/detorsion in rats



M. Ghasemnejad-Berenji[1,2,3] | M. Ghazi-Khansari[1] | I. Yazdani[1,2] | M. Nobakht[4] | A. Abdollahi[5] | H. Ghasemnejad-Berenji[6] | J. Mohajer Ansari[4] | S. Pashapour[7] | A. R. Dehpour[1,2]

[1]Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

[2]Experimental Medicine Research Center, Tehran University of Medical Sciences, Tehran, Iran

[3]Department of Pharmacology, Faculty of Pharmacy, Urmia University of Medical Sciences, Urmia, Iran

[4]Department of Anatomy, School of Medicine, Iran University of Medical Sciences, Tehran, Iran

[5]Department of Pathology, Cancer Institute, Tehran University of Medical Sciences, Tehran, Iran

[6]Department of Anatomy and Reproductive Biology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

[7]Department of Pathology, Urmia University of Medical Science, Urmia, Iran

Correspondence
Ahmad Reza Dehpour, Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran.
Emails: dehpour@yahoo.com, dehpourar@tums.ac.ir

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Summary

The study was designed to evaluate the effects of metformin on apoptosis and epididymal sperm quality in a rat testicular ischaemia/reperfusion (I/R) injury model. A total of 72 male rats were divided into four groups ($n = 18$ for each group): group 1 (sham-operated group), group 2 (metformin group), group 3 (torsion/detorsion [T/D] + saline) and group 4 (T/D + 300 mg kg^{-1} metformin). Testicular torsion was achieved by rotating the right testis 720° in a clockwise direction for 1 hr. Tissue malondialdehyde (MDA) level and caspase-3 activity increased and the activities of catalase, superoxide dismutase and glutathione peroxidase decreased in comparison with sham-operated group 4 hr after detorsion ($p < .001$). In six rats of each group 24 hr after detorsion, histopathological changes and germ cell apoptosis were significantly deteriorated by measuring mean of seminiferous tubule diameters (MSTD) and TUNEL test. Moreover, 30 days after T/D, sperm concentration and motility were examined in six animals per group. Metformin pre-treatment reduced MDA and caspase-3 levels and normalised 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. Reduction in oxidative stress and apoptosis may have a major role in cytoprotective effects of metformin.

KEYWORDS

apoptosis, metformin, oxidative stress, testicular torsion/detorsion

1 | INTRODUCTION

Testicular torsion by rotation of testis around the axis of the spermatic cord is a urologic pathologic condition that mainly affects newborns, children and adolescent boys, and inappropriate treatment can lead to infertility (Ergur, Kiray, Pekcetin, Bagriyanik, & Erbil, 2008; Turner & Brown, 1993). Rapid diagnosis and immediate surgical treatment are essential to prevent testicular damage (Perrotti, Badger, Prader, & Moran, 2006). The main pathophysiologic event in testicular torsion/detorsion is I/R injury of the testis (Turner & Brown, 1993), which stimulates an intracellular signalling cascade in the testicular endothelial cells that results in neutrophil recruitment, oxidative stress and germ cell-specific apoptosis (Lysiak, Nguyen, Kirby, & Turner, 2003; Lysiak et al., 2001; Turner, Tung, Tomomasa, & Wilson, 1997). Previous studies showed that I/R induces the loss of spermatogenesis mainly through germ cell-specific apoptosis (Turner et al., 1997). Apoptosis is a form of

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cell death characterised by organised nuclear and cellular fragmentation and has recently generated great interest in several areas of investigation, including infertility. In addition, in ischaemia phase, oxidative stress and germ cell apoptosis have important roles in testicular defects such as testosterone withdrawal, cryptorchidism and exposure to toxins (Shaha, Tripathi, & Mishra, 2010). Indeed, it has been suggested that inhibition of germ cell apoptosis may have a protective effect in testicular torsion (Mogilner et al., 2006; Turner et al., 1997). So, recognising therapeutic agents for preventing apoptosis in T/D condition would potentially be useful. Metformin (*N*,*N*-dimethylbiguanide) is the first-line drug for type 2 diabetes, particularly in overweight and obese people (Correia et al., 2008). Metformin induces AMP-activated protein kinase (AMPK), which is a key regulator of cellular energy balance (Zhou et al., 2001). Anti-inflammatory function of the metformin depends on its ability to inhibit the activation of NF-κB and enhancing the activation of AMPK (Kim & Choi, 2012; Li et al., 2009). AMPK is an upstream kinase of mammalian target of rapamycin (mTOR), and also an inhibitor of the mTOR pathway (Inoki, Kim, & Guan, 2012). Several studies have indicated that metformin may be useful against ischaemic injury in organs such as the liver, kidney, heart and brain (Ashabi, Khalaj, Khodagholi, Goudarzvand, & Sarkaki, 2015; Ashabi, Khodagholi, Khalaj, Goudarzvand, & Nasiri, 2014; Bhamra et al., 2008; Cahova et al., 2015; Seo-Mayer et al., 2011). Furthermore, it has been shown that the administration of metformin may also be protective against I/R injury in the testis in rat models (Asghari, Akbari, Meghdadi, & Mortazavi, 2016); however, there is no study about its effect on sperm quality and apoptosis in experimental testicular ischaemia/reperfusion. In the present study, for the first time we investigated the effect of metformin on germ cell apoptosis, oxidative stress and sperm quality in testicular I/R.

2 | MATERIAL AND METHODS

2.1 | Animals and experimental groups

Seventy-two male Wistar albino rats at 90 days of age weighing 230–260 g were used in this study. Animals were purchased from Department of pharmacology, Tehran University of Medical Sciences. They were maintained and used in accordance with the Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals prepared by the Tehran University of Medical Sciences Animal Ethical Committee. They were kept in an environment of controlled temperature (21–22°C), humidity (55%–60%) and controlled photoperiod (12/12 hr light:dark) for 1 week before the start of the experiment. The rats were randomly divided into four groups (n = 18 per group):

Group 1: Sham-operated rats, all the surgical steps were performed; however, T/D was not induced. The animals were kept under anaesthesia for the duration of the procedure.

Group 2: a single dose of metformin (300 mg kg^{-1}) was injected intraperitoneally (i.p.) and immediately surgical procedure was performed as in sham group;

Group 3: T/D-operated rats, received totally 2 ml injections of saline, 30 min before detorsion;

Group 4: T/D-operated rats received single dose of 300 mg kg^{-1} (i.p.) injection of metformin 30 min before detorsion (Payabvash, Kiumehr, Tavangar, & Dehpour, 2008; Yazdani et al., 2016).

2.2 | Experimental testicular T/D procedure

The rats were anesthetised with xylazine/ketamine (10/90 mg kg^{-1}, i.p.) under sterile conditions. The right testis entered through a midline incision and T/D was induced by rotating the right testis 720° in a clockwise direction for 1 hr. Then, the testis was counter-rotated to the natural position and was inserted into the scrotum, skin incision was sutured (4–0 nonabsorbable), and animals were kept until harvesting time. In the sham-operated animals, only surgical stress was occurred by immediately retraction and replacement of the spermatic cord. The biochemical markers of oxidative stress are reported to increase just 4 hr after reperfusion in testicular T/D model; thus, we removed the right testes of six animals from each study group 4 hr after detorsion for evaluation of oxidant/antioxidant balance. The testes of six other animals from each group were removed 24 hr after detorsion for determination of germ cell apoptosis index, which peaks at this time point. We also evaluated the effects of treatment on sperm count in six rats per each study group 30 days after treatment (Payabvash et al., 2008; Yazdani et al., 2016).

2.3 | Biochemical assays

Four hours after detorsion, ipsilateral orchiectomy was performed and samples were rapidly stored in −80°C for evaluation of tissue malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and caspase-3 level changes.

2.4 | MDA assay

Malondialdehyde accumulation in tissues is indicative of the extent of lipid peroxidation. MDA is an end product of peroxidation of fatty acid in the lipid peroxidation process. MDA levels in tissue homogenate were measured with the thiobarbituric acid reaction as described by Ohkawa, Ohishi, and Yagi (1979). In brief, testes were homogenised in 1.15% KCl to make a 10% (w/v) homogenate. Then, 0.9 ml of 1.8% sodium dodecyl sulphate (SDS), 1.5 ml of 20% acetic acid solution (pH = 3.5) and 1.5 ml of aqueous TBA solution were regularly added to 0.1 ml of tissue homogenates. The prepared homogenates were centrifuged at 4043 g for 10 min. The organic layer was taken and its absorbance at 532 nm was measured.

2.5 | CAT activity

Tissue catalase activity was spectrophotometrically measured according to method of Aebi (1984). Tissue sections were homogenised in 1% Triton X-100 and were diluted with potassium phosphate buffer. The reaction was initiated by the addition of hydrogen peroxide (H_2O_2)

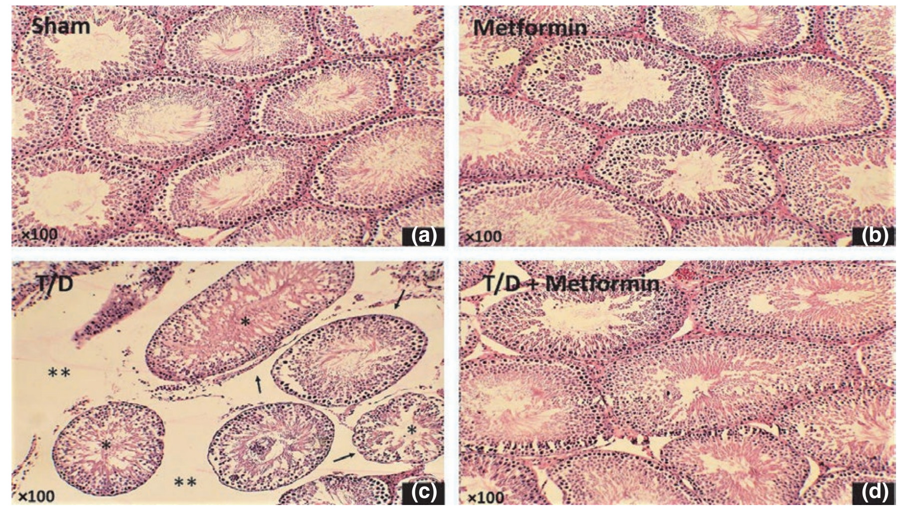

FIGURE 1 Light microscopy of testicular tissue in experimental groups. (a) Normal testicular histology in sham group. (b) Normal testicular histology in metformin group. (c) Sloughing (→), necrosis in interstitium (**) and decreased cellularity (*) in tubules in T/D group. (d) Increased cellularity with well-organised tubules and improved tubular structure with well-preserved epithelium

to reaction mixture, and the level of enzyme activity was quantitated according to ability of tissue catalase to decompensate H_2O_2 by monitoring decrease in absorbance at 240 nm.

2.6 | GPx activity

Glutathione peroxidase activity was measured by modified method of Paglia and Valentine (1967). The enzymatic reaction was initiated following addition of H_2O_2. In the assay, oxidised glutathione is reduced to glutathione by the enzyme glutathione reductase, which oxidises nicotinamide adenine dinucleotide phosphatase, reduced form (NADPH) to NADP in the catalytic cycle. The alteration in absorbance at 340 nm resulting from the oxidation of NADPH is the basis for quantitating tissue GPx activity.

2.7 | SOD activity

Tissue SOD activity was measured according to the Paoletti and Mocali method (Paoletti & Mocali, 1990). In brief, SOD activity level was assayed based on its ability to inhibit NADH oxidation in reaction mixture and conversion of superoxide anions (O_2) to H_2O_2 and molecular oxygen (O_2). SOD activity was measured by decrease in absorbance at 340 nm during the reaction.

2.8 | Caspase-3 level

Caspase-3 level was evaluated using ELISA detection kit according to the Biotin double-antibody sandwich technology. The colorimetric change in samples at 450 nm was applied to measure caspase-3 concentration (ng ml^{-1}) by drawing a standard curve (Namura et al., 1998).

2.9 | Histopathological analysis

The testes of six other rats from each study group were removed 24 hr after detorsion following a rapid cervical dislocation. The specimens were fixed in 10% phosphate-buffered formalin and post-fixed in 70% ethanol, and then were cut in 5-μm-thick sections. After deparaffinisation of the sections and staining with haematoxylin–eosin (H&E), the tissues were examined under light microscope with 100× magnification to quantify testicular histological injury (Figure 1). The four-level grading scale of Cosentino's score was used (Cosentino, Nishida, Rabinowitz, & Cockett, 1986):

Grade 1: normal structure with regular arrangement of germ cells;
Grade 2: testicular injuries with less orderly, noncohesive germ cells and closely packed seminiferous tubules;
Grade 3: testicular injuries with disordered, sloughed germ cells with shrunken, pyknotic nuclei and less distinction in seminiferous tubule borders;
Grade 4: testicular injuries with coagulative germ cell necrosis and intensely packed seminiferous tubules.

Moreover, for each sample, MSTD was calculated by measurement of 10 separate roundest seminiferous tubules using a light microscope-adaptable micrometre.

2.10 | Evaluation of germ cell apoptosis using TUNEL assay

For quantitative assessment of apoptosis, apoptotic nuclei in tissue sections were identified with the in situ terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate–biotin nick end labelling (TUNEL) technique, which identified DNA strand breaks by labelling their free 3′-OH termini (Negoescu et al., 1996). Semiquantitative assessment of apoptotic nuclei in specimens was performed using the APO-BrdU-IHC kit according to the manufacturer's instructions. About 100 seminiferous tubule cross sections from each specimen were evaluated for appearance of apoptotic nuclei with intense green staining by manual counting at 200× magnifications (Figure 2).

The mean number of TUNEL-positive cells per tubule cross section was obtained, and only circular tubular cross sections cut in boldface were evaluated (Payabvash et al., 2008; Yazdani et al., 2016).

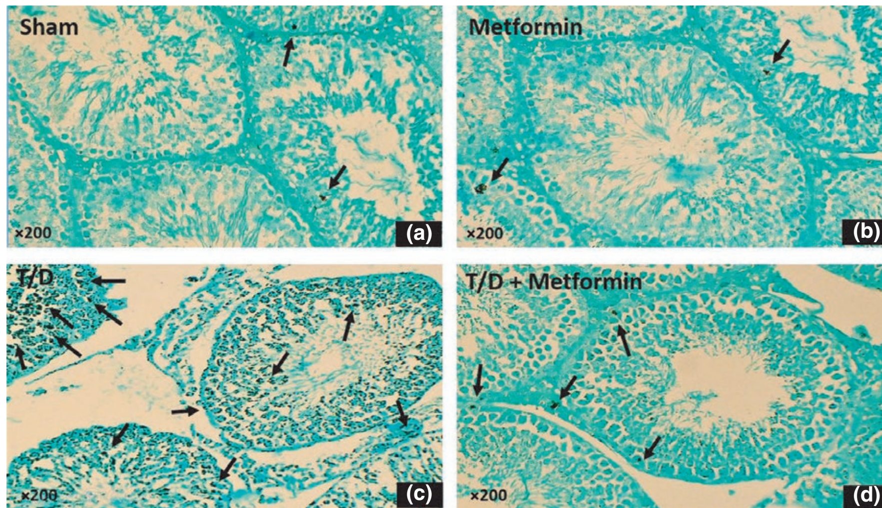

FIGURE 2 Effects of T/D and metformin on germ cell apoptosis and TUNEL immunoreactivity in testis tissue. (a) The sham group shows a few TUNEL-positive cells. (b) The metformin group revealed fewer TUNEL-positive cells in testis as in the sham group. (c) After T/D, TUNEL-positive cells markedly decreased in the seminiferous tubules. (d) TUNEL-positive cells decreased in the T/D + metformin group

2.11 | Semen functional analysis

Long-term effects of T/D and treatment with metformin (300 mg kg^{-1} i.p.) were evaluated by determination of changes in sperm concentration and motility 30 days after T/D operation. The epididymal sperm concentration was measured by Yokoi et al. method (Yokoi, Uthus, & Nielsen, 2003). In brief, after killing the animals, epididymis was completely removed, finely minced in physiological saline and incubated at room temperature for 5 min after 20-min shaking. Then, supernatant fluid was diluted and dyed in proportion 1:100 with a solution containing 5 g sodium bicarbonate, 1 ml 35% formalin and 25 mg eosin (in 100 ml of water). The solution was centrifuged at 5071 g for 20 min at room temperature. Under light microscopy, spermatozoa were counted following transfer of approximately 10 µl of the diluted sperm suspension to each counting chamber of hemocytometer (200× magnification). Using a standard method which possesses a score ranging from 0% to 100%, the percentage of progressive sperm motility was visually recorded (400× magnification). Final motility percentages were calculated by mean value of four specific estimations in each sample.

2.12 | Statistical analysis

All data were expressed as mean ± *SD*. The differences between the experimental groups were analysed by ANOVA. Individual groups were compared using Tukey's multiple comparison tests. Differences less than 0.05 were considered statistically significant ($p < .05$).

3 | RESULTS

3.1 | Biochemical assays

The results of testicular MDA concentration and SOD, CAT, GPx and caspase-3 activities in studied groups are shown in Table 1. There were significant differences in the evaluated antioxidant enzymes levels between the T/D- and sham-operated group. The tissue MDA concentration in the T/D + metformin (300 mg kg^{-1}, i.p.) was significantly lower than T/D animals ($p < .001$). It is proven that treatment with metformin (300 mg kg^{-1} i.p.) exerted its protective effects by a reduction in lipid peroxidation induced by I/R. The activity of SOD, CAT and GPx enzymes in the T/D rats significantly increased following injection of metformin ($p < .001$). Furthermore, treatment with metformin reduced caspase-3 activity in ischaemic/reperfused tissue ($p < .001$).

3.2 | Histopathological analysis

The histopathological parameters in right testes among the four experimental studied groups are demonstrated in Table 2. When the structure of testis was histopathologically examined, it was

TABLE 1 Testicular levels of MDA and CAT, SOD, GPx and caspase-3 enzyme activities 4 hr after detorsion

Group	MDA (nmol/g wet issue)	CAT (IU/g wet tissue)	SOD (IU/g wet tissue)	GPx (IU/g wet tissue)	Caspase-3 activity (ng/ml)
Sham	113.15 ± 6.13	364.19 ± 4.94	1,967.23 ± 10.71	731.53 ± 52.26	0.264 ± 0.027
Metformin	107.24 ± 21.43	371.62 ± 13.02	1,998.39 ± 19.78	748.29 ± 28.98	0.233 ± 0.014
T/D	194.02 ± 11.15*,**	221.31 ± 16.53*,**	1,495.15 ± 25.01*,**	512.24 ± 31.99*,**	0.467 ± 0.021*,**
T/D + Metformin	121.46 ± 14.15	305.21 ± 20.63	1,921.58 ± 50.34	691.47 ± 12.54	0.305 ± 0.027

T/D, torsion/detorsion; MDA, malondialdehyde; SOD, superoxide dismutase; CAT, catalase; GPx, glutathione peroxidase.
*$p < .001$ compared with sham group.
**$p < .001$ compared with T/D + metformin group.

TABLE 2 Histological evaluation of the testes using mean seminiferous tubular diameter (MSTD) values and Cosentino's scores 24 hr after detorsion in the studied rats

Group	MSTD (μm)	Grade
Sham	288.8 ± 41.32	1
Metformin	285.3 ± 8.48	1
T/D	215.5 ± 32.11*,**	3
T/D + metformin	261.3 ± 5.81	2

Grades: 1, minimal or no evidence of injury; 2, slight injury; 3, mild injury; 4, moderate injury.
*$p < .001$ compared with sham group.
**$p < .001$ compared with T/D + metformin group.

observed that the histological appearance of testicular tissues of groups 1 and 2 was normal. As expected, the architecture of the seminiferous tubules, germ cells, Sertoli and Leydig cells appears complete in sham-operated animals (group 1) and group 2 without infiltration and haemorrhagic sign. Testicular torsion caused different histopathological features, and significant ($p < .001$) decreases in MSTD and increased Cosentino's scores were observed in the testis of group 3 compared with the groups 1 and 2 (Table 2). These histopathological changes were significantly less obvious in group 4.

3.3 | TUNEL assay

Immunohistochemical studies confirm the index of germ cells following TUNEL assay (Figure 2). By double labelling, alterations in the anatomical structures and proportion of the TUNEL-positive nuclei/surrounding normal nuclei (%) were determined. Only those TUNEL-positive cells that fulfilled the morphological criteria for apoptosis (i.e., cell shrinkage, nuclear fragmentation and condensation) were counted. Germ cell apoptosis indices were significantly higher in the T/D and metformin group versus the sham-operated group (Table 3); however, injection of metformin reduced the apoptosis index. According to the Immunohistochemical studies, treatment with metformin (300 mg kg^{-1} i.p.) reduced the apoptosis in T/D + metformin group (group 3) compared with T/D group.

TABLE 3 Apoptotic germ cell index and percentage of apoptotic tubules in the rat testes determined using TUNEL assay

Group	Mean apoptotic nuclei/tubule	Apoptotic tubules (%)[a]
Sham	0.76 ± 0.57	13.28 ± 2.354
Metformin	0.71 ± 0.19	11.67 ± 1.149
T/D	8.49 ± 0.39*,**	61.51 ± 2.235*,**
T/D + metformin	3.07 ± 1.52	31.41 ± 3.261

[a]The percentage of tubules in each specimen in which at least 1 TUNEL-stained nucleus is observed.
*$p < .001$ compared with sham group.
**$p < .01$ compared with T/D + metformin group.

3.4 | Semen analysis

The results demonstrated the alterations in sperm concentration and motility due to testicular T/D (Figure 3). Injection of metformin (300 mg kg^{-1}) significantly improved the reduced epididymal sperm concentration induced by testicular T/D ($p < .001$). Furthermore, the percentage of sperm motility resulted from testicular T/D was increased following treatment with metformin at dose of 300 mg kg^{-1} ($p < .01$).

4 | DISCUSSION

The present study demonstrated the ability of metformin to improve sperm quality in ipsilateral testis undergoing unilateral testicular T/D. Based on our study, metformin administration ameliorated testicular tissue damage and improved epididymal sperm concentration and motility through reduction in oxidative stress and germ cell apoptosis.

I/R injury is the detrimental pathological consequence of testicular T/D, which induces the overproduction of free radicals such as Reactive oxygen species (ROS) and impairs the equilibrium between oxidants and antioxidants (Akçora et al., 2008). These pathologic pathways which are known as oxidative stress cause lipid peroxidation and trigger a chain of events that can cause cellular dysfunction and promote apoptosis (Chandra, Samali, & Orrenius, 2000). MDA, the end product of lipid peroxidation, is a well-known parameter for evaluating the increased free radical formation in post-ischaemic tissue (Takhtfooladi et al., 2013). SOD, CAT and GPx are major enzymes that scavenge harmful ROS in male reproductive organs (Pekcetin et al., 2007). Previous studies have shown that I/R leads to increased MDA and inactivation of antioxidant enzymes in rat testes (Salmasi et al., 2005; Takhtfooladi et al., 2013). Therefore, determination of the therapeutic practices for rescuing the testis from I/R injury by protecting it from all forms of oxidative stress would potentially be useful. Recently, a number of chemical agents and drugs such as oxygen radical scavengers have been successfully used to reduce the I/R injury in animal models of testicular torsion. But only few of them are currently in clinical use because of severe adverse effects (Celik et al., 2016; Dokmeci et al., 2007; Jahromi, Rasooli, Kamali, Ahmadi, & Sattari, 2017; Meštrović et al., 2014, 2016; Zavras et al., 2014).

Metformin as a antidiabetic biguanide agent improves the sensitivity to insulin, increases the insulin-stimulated uptake and utilisation of glucose, reduces basal hepatic glucose production, causes weight reduction and decreases hunger (Rena, Pearson, & Sakamoto, 2013). This drug is generally well tolerated, and the most common adverse effect of metformin is gastrointestinal irritation (Bolen et al., 2007; Triggle & Ding, 2016). Metformin induces AMPK, which is a key regulator of cellular energy balance (Zhou et al., 2001). The activation of AMPK signalling pathway during I/R has been considered to be an endogenous compensatory mechanism to protect against oxidative stress injury (Kim, Kundu, Viollet, & Guan, 2011; Russell et al., 2004). Metformin exerts its effect through both AMPK-dependent and AMPK-independent mechanisms (Sahra et al., 2008).

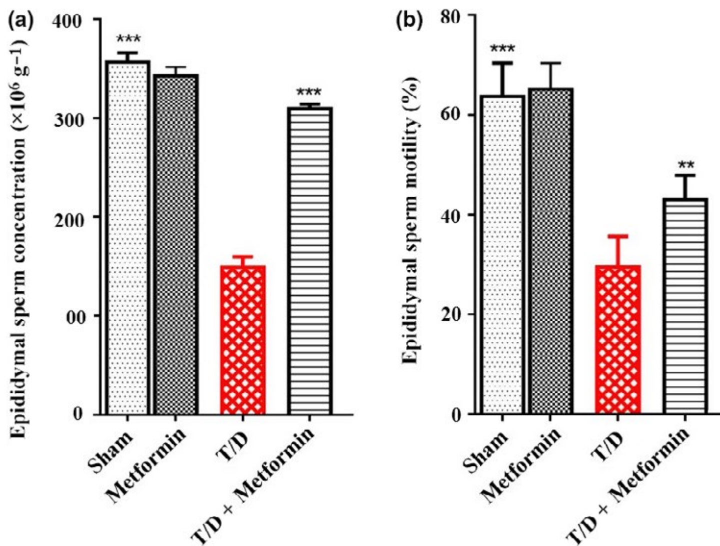

FIGURE 3 Graph (a) showing sperm count and graph (b) showing sperm motility in all groups at 30 days of reperfusion. $^{**}p < .01$; $^{***}p < .001$ compared with T/D

Recent studies have shown that metformin can decrease cell apoptosis in tissues like liver, muscle and kidney (Viollet & Foretz, 2013; Vytla & Ochs, 2013; Wang et al., 2015). Recently, the protective role of metformin has been demonstrated in the brain of rats following cerebral I/R injury (Abd-Elsameea, Moustaf, & Mohamed, 2014). In another study, Cahova et al. showed that metformin administration prevents ischaemia/reperfusion-induced oxidative stress in the fatty liver by attenuation of ROS formation. Our results are consistent with these studies (Cahova et al., 2015). We showed that pre-treatment with metformin was associated with reduced MDA accumulation in the induced testicular T/D tissue, which indicates the reduction in lipid peroxidation reaction rate. Fuse et al. showed the sperm counts in the left caudal epididymis were significantly reduced after ischaemia and sperm motility also decreased after the procedure in rats (Fuse, Tsuritani, Iwasaki, & Katayama, 1992). Yazdani et al. (2016) reported that 30 days after 1-hr torsion in rats both sperm count and motility were reduced. Similarly, our data suggest that 1-hr ischaemia causes decreased sperm concentration and sperm motility 30 days after ischaemia. In the present study, T/D caused a significant increase in disorganisation, degeneration and desquamation in germinal cells and interstitial oedema in testis and also reduced the histological parameters such as MSTD. The detrimental effects of I/R on sperm concentration may imply an interference with spermatogenesis. Additionally, ischaemia could induce apoptotic degeneration of testicular tissues, which may contribute to spermatogenesis loss in the testis. The slump in sperm concentration may be due to IR-induced oxidative stress in testicular tissue. Oxidative stress plays a crucial role in the aetiology of defective testis function via mechanisms involving the induction of peroxidative damage to the plasma membrane (Agarwal & Saleh, 2002; Vernet, Aitken, & Drevet, 2004; Visser & Heyns, 2003).

Metformin pathways in protecting cells against oxidative stress are manifold; first, metformin could directly engage with ROS in vitro. In a study by Bonnefont-Rousselot et al. (2003), metformin was able to scavenge hydroxyl free radicals. Similar results were reported by Hou et al. (2010) in investigation of the reduction in intracellular ROS in endothelial cells treated with metformin. Second, metformin is capable of abating ROS by inhibition its production at intracellular level. This is achieved by either by reducing NAD(P)H, or to a lesser extent by decreasing respiratory chain reactions in mitochondria (Ouslimani et al., 2005).

Based on our records, metformin administration ameliorated testicular tissue damage and improved epididymal sperm concentration and motility through reduction in oxidative stress and germ cell apoptosis after T/D injury. Apoptotic cells were determined using a TUNEL assay based on the detection of DNA strand breaks that occur during apoptosis (Yazdani et al., 2016). The important role of germ cell apoptosis in the normal testicular function has been reported in several studies (Modi, Sane, & Bhartiya, 2003). Despite physiological testicular function, studies have shown that the enhancement of germ cell apoptosis is a responsible mechanism of impaired spermatogenesis and infertility in testicular torsion (Hadziselimovic, Geneto, & Emmons, 1998). Yazdani et al. (2016) observed TUNEL-positive germ cells in the seminiferous epithelium of I/R-injured testis tissue. Previous studies have reported the potency of metformin in reducing ROS, and scavenging hydroxyl free radicals and preventing lipid peroxidation, an autocatalytic mechanism, which leads to oxidative damage of cell membranes and, consequently, cell death by apoptosis (Viollet et al., 2012). In the present study, we demonstrate increased numbers of TUNEL-positive (apoptotic) cells after I/R. Treatment with metformin significantly reduced the number of apoptotic cells, decreased the MDA and caspase-3 level and increased the activity of antioxidant enzymes in the rats undergoing testicular T/D. This is in consistent with previous reports, which demonstrate that metformin scavenges ROS, inhibits lipid peroxidation and maintains antioxidant defences during oxidative stress (Bonnefont-Rousselot et al., 2003; Viollet et al., 2012).

In conclusion, our findings reveal that administration of metformin reduces cellular injury depicted by lower levels of MDA and apoptosis indices compared to T/D group and leads to later improvement in sperm count and motility. The antioxidant and anti-apoptotic properties of metformin have possibly contributed to these effects. Consequently, metformin, as an adjunctive drug in testicular detorsion operation, may have a useful approach to reduce surgical reperfusion injuries and could improve spermatogenesis in injured testes.

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