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Original Article

Effects of Magnesium Sulfate Administration on Testicular Ischemia/Reperfusion Injury in Rats

Moshkelani [1], S., Asghari [1]*, A., Abedi [1], G., Jahandideh [1], A., Mortazavi [2], P.

1. Department of Clinical Sciences, Science and Research Branch, Islamic Azad University, Tehran, Iran
2. Department of Pathobiology, Science and Research Branch, Islamic Azad University, Tehran, Iran

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Corresponding Author: dr.ahmad.asghari@gmail.com

ABSTRACT

This study aimed at investigating the effects of intraperitoneal (IP) administration of magnesium sulfate ($MgSO_4$) on testicular ischemia-reperfusion (IR) injury in rats. In total, 50 adult Wistar rats were randomly divided into 5 groups. Group 1 received no injection (control); however, group 2 was subjected to 2 h of I and 24 h of R. Subsequently, group 3 was subjected to 2 h of 1, and after 1 h of I, 125 mg/kg $MgSO_4$ was injected intraperitoneally followed by 24 h of R. Groups 4 and 5 were subjected to the same process as group 3, whereas the rats were injected with 250 and 500 mg/kg of $MgSO_4$, respectively. After 24 h, the left testes of all rats were removed for histological analysis and antioxidant activities. According to the results, there was a significant increase in tissue malondialdehyde (MDA) among I/R rats ($P<0.05$), whereas $MgSO_4$ decreased I/R-induced MDA ($P<0.05$). Furthermore, experimental I/R diminished glutathione peroxidase (GPx) and superoxide dismutase (SOD) levels significantly ($P<0.05$). Moreover, $MgSO_4$ (250 and 500 mg/kg) increased GPx and SOD activity significantly in I/R rats ($P<0.05$). Furthermore, seminiferous tubules degenerated, and few spermatocytes were observed in the testis tubules of the I/R rats. Regarding pathological parameters, seminiferous tubules and spermatocyte were normal in the testes of $MgSO_4$ (250 and 500 mg/kg)-treated experimental I/R-induced rats. In conclusion, this study demonstrated the beneficial effects of $MgSO_4$ on testicular IR injury in rats.

Keywords: Ischemia/reperfusion (I/R), Magnesium sulfate, Rat, Testis

|Les Effets de l'Administration de Sulfate de Magnésium sur l'Ischémie Testiculaire/Lésion de Reperfusion chez Le Rat

Résumé: Cette étude visait à étudier les effets de l'administration intrapéritonéale (IP) de sulfate de magnésium ($MgSO_4$) sur les lésions d'ischémie-reperfusion (IR) testiculaire chez le rat. Au total, 50 rats Wistar adultes ont été répartis au hasard dans 5 groupes. Le groupe 1 n'a reçu aucune injection (témoin); alors que le groupe 2 a été soumis à 2 h de I et 24 h de R.Ensuite, le groupe 3 a été soumis à 2 h de I suivi d'une heure de I. Un total de125 mg/kg de $MgSO_4$ ont été injectés par voie intrapéritonéale, suivis de 24 h de R. Les groupes 4 et 5 ont été soumis au même protocole que le groupe 3, à la différence que les rats ont reçu respectivement 250 et 500 mg/kg de $MgSO_4$. Les testicules gauches de tous les rats ont été prélevés pour une analyse histologique et de l'activité antioxydantes après 24 h. Selon nos résultats, il y avait une augmentation significative du malondialdéhyde tissulaire (MDA) chez les rats I/R ($P<0.05$), tandis que le $MgSO_4$ diminuait la MDA induite par l'I/R ($P<0.05$). De plus, les I/R expérimentaux ont diminué de manière significative les niveaux de glutathion peroxydase (GPx) et de superoxyde dismutase (SOD) ($P<0.05$). En outre, le $MgSO_4$ (250 et 500 mg/kg) a augmenté significativement l'activité GPx et SOD chez les rats I/R ($P<0.05$). De plus, les tubules séminifères ont dégénéré et peu de spermatocytes ont été observés dans les tubules testiculaires des rats I/R. Les tubules séminifères et les spermatocytes étaient normaux concernant les paramètres pathologiques dans les testicules de rats

expérimentaux induits par I/R traités par $MgSO_4$ (250 et 500 mg/kg). En conclusion, cette étude a démontré les effets bénéfiques du $MgSO_4$ sur les lésions IR testiculaires chez le rat.
Mots-clés: Ischémie/reperfusion (I/R), Sulfate de magnésium, Rat, Testicule

INTRODUCTION

Testicular torsion is a urologic emergency that leads to serious infertility (Parlaktas et al., 2014). It can happen in children and young males, thereby requiring urgent diagnosis and treatment (Celik et al., 2016). The duration and degree of twisting of the cord are closely related to the severity of the testicular injury (Aydıner et al., 2012). The testicular torsion is defined as a circulatory failure by testis revolving around the vascular peduncle (Yulug et al., 2013). The main pathophysiologic event in testicular torsion is ischemia followed by reperfusion (Pogorelic et al., 2016). Therefore, testicular torsion detorsion is an ischemia/reperfusion (I/R) injury for the testis (Asghari et al., 2018a). Diminished blood flows occur in ipsilateral and contralateral internal spermatic arteries after unilateral torsion. These changes result in testicular function impairment and fertility (Yulug et al., 2013). Interruption in tissue blood supply by I/R leads to cellular and tissue damage (Koksal et al., 2012). Testicular torsion terminates to tissue degeneration and usually requires emergency surgical intervention for the reperfusion of the affected testis (Celik et al., 2016). There are numerous reports on medications and/or interventions utilized to manage this condition. In physical therapy, hyperbaric oxygen (through plasma oxygen transport), hypothermia, and ischemic post-conditioning are being used as surgical techniques against reperfusion injury for the first-line treatment (Arena et al., 2017). Among the medications, agonists of erythropoietin receptors, dexmedetomidine, morphine, and antioxidants, such as dimethylsulfoxide, zinc, vitamin E, melatonin, and plant antioxidant extracts are widely suggested by the clinicians (Arena et al., 2017). The exact pathological mechanisms of testicular torsion causing the injury are not fully elicited; however, the excessive reactive oxygen species (ROS) generation impairs spermatozoal motility (Eghbali et al., 2010). The ROS has a deniable effect on male infertility. Reperfusion injury is related to the elevated of free oxygen radicals which causes cell membrane lipid peroxidation and DNA impairment (Raju et al., 2011). Epididymal antioxidant enzymes protect spermatozoa from oxidative damage in the epididymal lumen (Fakouri et al., 2017). Magnesium (Mg^{2+}) is the second plentiful cation within the cell and has a key role in various physiologic actions, including cell cycle, ATPase activity, channel regulation, and metabolic regulation (Romani, 2007). The Mg^{2+} deficiency is related to oxidative stress in pathologic circumstances (e.g. diabetes, hypertension, atherosclerosis, and neuronal injury) (Wolf et al., 2009). Moreover, it is reported that ROS-mediated DNA damage is related to Mg^{2+} dependent inhibition of cell growth (Wolf et al., 2009). Furthermore, Mg^{2+} has been revealed to prevent lipid peroxidation and reduce neuroprotective effects in I/R injury of the fetal rat brain (Hasturk et al., 2013). In addition, Mg^{2+} protects livestock spermatozoa against freezing and thawing injury (Pesch et al., 2006). The $MgSO_4$ diminishes superoxide dismutase (SOD) in the bile duct ligation-induced in rats (Eshraghi et al., 2015). The imbalance between ROS and scavenge free antioxidants occurs during oxidative stress (Agarwal et al., 2009). The ROS are crucial requirements of spermatozoa for fertilization, capacitation, motility, and acrosomal reaction (Hadwan et al., 2014). Although there is a report on the correlation between seminal

antioxidant and Mg^{2+} levels with sperm viability and motility (Pesch et al., 2006), no studies investigated the effects of $MgSO_4$ on testicular IR injury in rats. Therefore, this study aimed to evaluate the effects of $MgSO_4$ injection on testicular IR injury in rats.

MATERIAL AND METHODS

Animals. In total, 50 healthy adult male Wistar rats with a mean weight of 270 g were purchased from the Pasteur Institute and kept in constant room temperature (20±1°C), relative humidity (42±1%), and 12-hour light/ dark cycle. Subsequently, they were randomly divided into five experimental groups of 10 per group. All protocols for animal experiments were approved by the Institutional Animal Ethical Committee, Islamic Azad University, Science and Research Branch, Tehran, Iran (Ethic code: 25876).

Chemicals. The $MgSO_4$ was purchased from Sigma Chemicals (Poole, Dorset, UK); in addition, the assay kits of malondialdehyde (MDA), SOD, and glutathione biosynthesis (GPx) were obtained from Randox Laboratories (Ltd., Crumlin, Antrim, United Kingdom). The $MgSO_4$ dosages were selected based on previous reports (Eshraghi et al., 2015; Asghari et al., 2018b).

Experimental protocol. Anesthesia was accomplished using an intraperitoneal injection of ketamine hydrochloride (60 mg/kg) and xylazine hydrochloride (10 mg/kg) during experimental testicular IR (Koksal et al., 2012). A midline longitudinal incision was made to have access to both testes. Torsion was created by twisting the left testis 720° in a counterclockwise direction and maintained by fixing the testis to the scrotum with a 6-0 nylon suture passing through the tunica albuginea and dartos. The suture was removed 2h post-ischemia, and the left testis was detorted and replaced with scrotum reperfusion continued for 24 h (Sahin et al., 2005). Group 1 was considered as control with no surgery, and group 2 was subjected to 2h of I and 24h of R. Moreover, group 3 was subjected to 2h of I, and after 1 h of I, the rats were injected intraperitoneally with 125 mg/kg $MgSO_4$ followed by 24h of R. In the similar vein, the rats in group 4 were subjected to 2h of I, and after 1 h of I, they were injected intraperitoneally with 250 mg/kg $MgSO_4$ followed by 24h of R. Group 5 was also subjected to 2h of I, and after 1 h of I, 500 mg/kg $MgSO_4$ was administered to the rats intraperitoneally followed by 24h of R. After 2 h of I, the suture was removed and the left testis detorted and replaced into scrotum for 24 h of reperfusion. At the end of the study, the rats were euthanized with pentobarbital (300 mg/kg, IP), the peritoneum was opened, and the left testis was removed. Following that, the testicle was divided into two halves using a sagittal section. The first half of the testicle tissue was fixed in Bouin's solution, and the second half was stored at -80 °C for the biochemical analysis (Fakouri et al., 2017). The right testis was removed as a control for histological investigations. Diagram 1 describes the entire study protocol.

Tissue processing. The tissue was fixed in 7.5 ml saturated picric acid, 2.65 ml glacial acetic acid, and 2.5 mL 7% formaldehyde (Bouin's solution); additionally, it was post-fixed in 70% alcohol and embedded in paraffin blocks. A 5µm tissue section was obtained, deparaffinized, and stained with Hematoxyline and Eosin. The testicular tissue was evaluated under light microscopy. In the next stage, the testis tissue was fixed at Bouin's solution for complete fixation and processed for paraffin sectioning. A tissue section about 5µm thickness was taken and stained with Hematoxyline and Eosin. The testis sections were graded based on the seminiferous tubule injury according to Johnsen (1970).

Antioxidant activity. The tissue MDA levels were determined by a method based on the reaction with thiobarbituric acid (Wasowicz et al., 1993) and maximum absorption at 532 nm (Placer et al., 1966). The GPx level was measured in the absorbance of 340 nm (Paglia and Valentine, 1967). The total antioxidant status kit was obtained on the basis of suppression in color production which was measured at 600 nm and

expressed as mmol/ml (Paoletti and Mocali, 1990; Miller et al., 1993).

Statistical analysis. The parametric data were analyzed in SPSS software (version 24) (SPSS, Inc., Chicago, IL, USA) through a one-way analysis of variance and expressed as mean±standard error. In case of heterogeneity occurrence, the groups were separated using Duncan Multiple Range Test. Moreover, the Kruskal-Wallis test was used to compare group medians for histopathological scores. A p-value less than 0.05 was considered statistically significant.

RESULTS

The I/R had the lowest testis damage grade, compared to other groups (P<0.05) (Figure 1).

On the other hand, the testis damage grade was the highest in the control group (P>0.05). A significant difference was observed between $MgSO_4$ treated groups and group 2 regarding testis damage grade (P<0.05). Moreover, no difference was observed among groups 3, 4, and 5 regarding the administration of $MgSO_4$ at different dosages (P>0.05). According to Table 1, there is a significant increase in the tissue MDA levels in group 2 (P<0.05), whereas the $MgSO_4$ dosages of 125, 250, and 500 mg/kg (groups 3-5) decrease I/R-induced MDA (P<0.05). Moreover, experimental I/R decreases GPx and SOD activity significantly (P<0.05); however, the injection of the $MgSO_4$ (125, 250, and 500 mg/kg) elevates SOD and GPx activity significantly (P<0.05). There is no significant difference among the experimental groups in terms of total antioxidant status (P>0.05). Furthermore, the left and right testis sections of group 1 reveal normal seminiferous tubules and

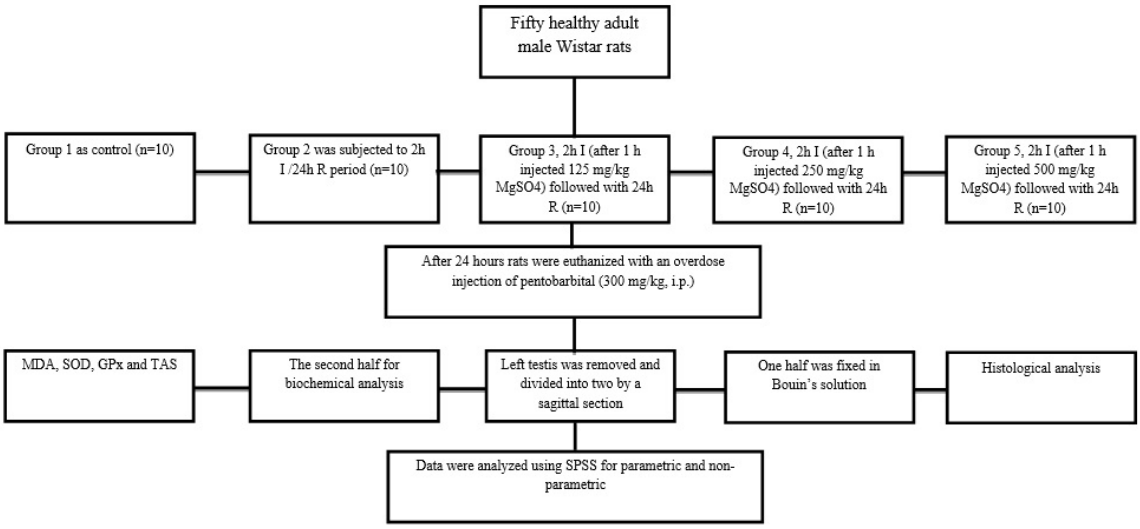

Diagram 1.

Table 1. Effect of different levels of $MgSO_4$ on tissue values of malondialdehyde, superoxide dismutase, glutathione peroxidase, and total antioxidant status in experimental testicular I/R-induced rats

Group	**MDA (nmol/g tissue)**	**SOD (U/mg tissue)**	**GPx (U/mg tissue)**	**TAS (mmol/ml)**
Control	103.11±2.12 [d]	4.12±0.14 [a]	4.25±0.15 [a]	15.04±1.81
I/R	180.17±2.10 [a]	1.89±0.18 [d]	2.43±0.20 [d]	12.09±1.54
$MgSO_4$ (125 mg/kg)	172.02±1.19 [b]	1.94±0.16 [c]	3.24±0.32 [c]	12.30±1.22
$MgSO_4$ (250 mg/kg)	147.12±1.30 [b]	2.31±0.18 [c]	3.33±0.12 [c]	13.01±1.14
$MgSO_4$ (500 mg/kg)	117.14±2.13 [c]	3.34±0.21 [b]	3.89±0.19 [b]	13.56±1.42

$MgSO_4$: magnesium sulfate, MDA: malondialdehyde, SOD: superoxide dismutase, GPx: glutathione peroxidase, TAS: total antioxidant status, I/R: ischemia/reperfusion. Different letters (a-d) indicate significant differences between treatments (P<0.05).

spermatogenesis with spermatocytes, Sertoli, and spermatozoa (Figure 2).

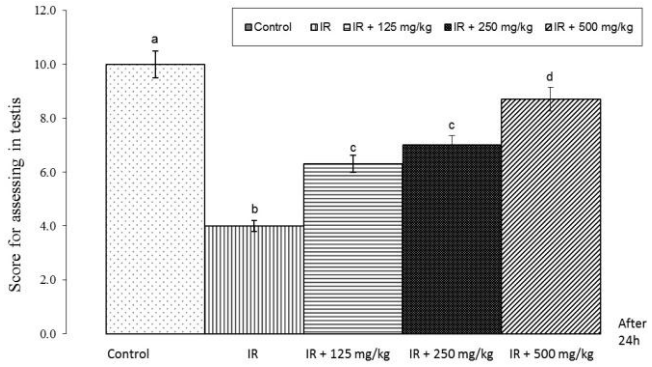

Figure 1. score of assessing in testis associated with seminiferous tubules injury in experimental I/R rat. Different letters (a-d) indicate significant differences between treatments ($P<0.05$).

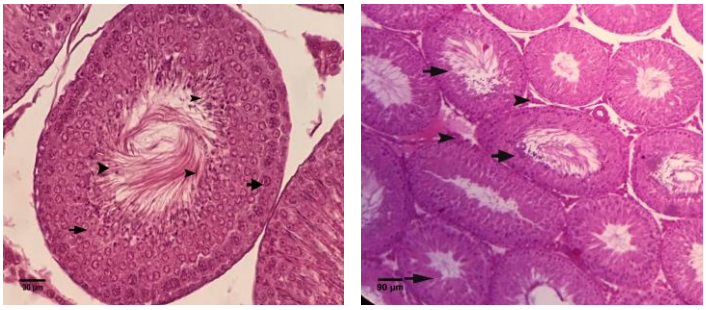

Figure 2. Testis section of left testis in control rats showing normal seminiferous tubules (Arrow) and interstitial cells (Arrow head) between tubules (Left). Testis section of right testis in control rats showing normal seminiferous tubules with spermatogonia (black arrow), spermatocyte (black arrow head) and many spermatozoa (white arrow) (Right). H & E: Hematoxylin and Eosin.

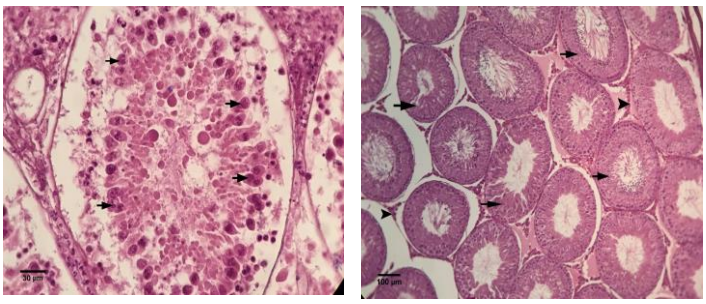

Figure 3. Testis section of left testis in I/R rats showing degenerated seminiferous tubules (arrow) and loss of spermatogenesis (H&E) (Left) and testis section of right testis in I/R rats showing normal seminiferous tubules (Arrow) and interstitial cells (Arrow head) between tubules (Right). H & E: Hematoxylin and Eosin.

Degeneration of seminiferous tubules and loss of spermatogenesis with few spermatocytes were observed in left degenerated testis tubules in group 2. However, there was no significant effect on the right testis (Figure 3). Furthermore, degeneration of seminiferous tubules and loss of spermatogenesis with few spermatocytes were observed on the left testis following the injection of the $MgSO_4$ (125 mg/kg) (Figure 4). However, no significant effect was observed on the right testis (Figure 4). As can be seen in Figure 5, the IP administration of the $MgSO_4$ (125 mg/kg) followed by I/R improved testis characteristics with few normal seminiferous tubules and spermatocyte in the testis of the rats in group 2. Moreover, the administration of $MgSO_4$ (500 mg/kg) improved testis characteristics with normal seminiferous tubules and spermatocyte in the testis of the rats in group 2 (Figure 6).

DISCUSSION

There is little evidence regarding the effects of $MgSO_4$ on male infertility and I/R injury. This is the first study that has determined the effects of $MgSO_4$ on semen MDA, SOD, and GPx in I/R injuries. As observed, $MgSO_4$ dependently decreased MDA and increased SOD and GPx activities in I/R-induced rats. Moreover, seminiferous tubules degenerated, and loss of spermatogenesis with few spermatocytes were detected in the degenerated testis tubules among I/R rats. In addition, the repeated succession of IR injury in testicular cells caused many biochemical and morphological changes which might lead to lipid peroxidation, protein denaturation, DNA damage, and apoptosis (Kanter, 2010). In the past few years, several anti-inflammatory antioxidants and free-radical scavengers were utilized for the treatment of testicular I/R, which induced male infertility. The $MgSO_4$ improved testis characteristics in experimental I/R-induced rats. The imbalance between ROS generation and the antioxidants leads to oxidative stress (Hwang and Lamb, 2012). It is reported that more than 60% of

male infertility happens via ROS mediated sperm damage (Agarwal et al., 2014).

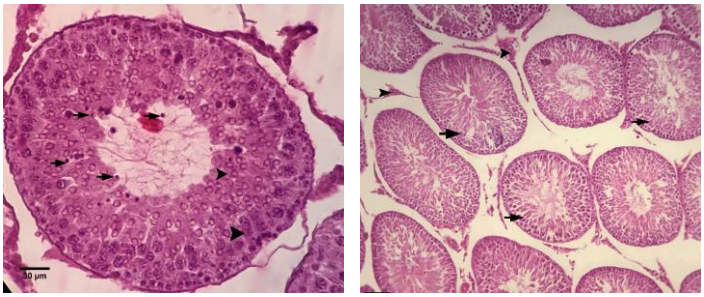

Figure 4. Testis section of left testis in the $MgSO_4$ (125 mg/kg) followed by I/R rats showing seminiferous tubules (Arrow) with few spermatocyte and interstitial cells (Arrow head) between tubules (Left) and testis section of right testis in the $MgSO_4$ (125 mg/kg) followed by I/R rats showing normal seminiferous tubules (Arrow) and interstitial cells (Arrow head) between tubules (Right). H & E: Hematoxylin and Eosin. $MgSO_4$: magnesium sulfate.

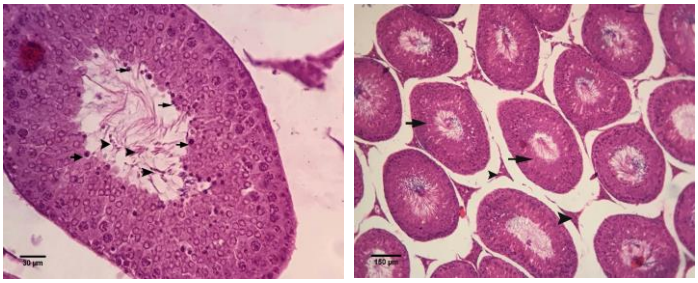

Figure 5. Testis section of left testis in the $MgSO_4$ (250 mg/kg) followed by I/R rats showing seminiferous tubules (Arrow) with few spermatocyte and interstitial cells (Arrow head) between tubules (Left) and testis section of right testis in the $MgSO_4$ (250 mg/kg) followed by I/R rats showing normal seminiferous tubules (Arrow) and interstitial cells (Arrow head) between tubules. H & E: Hematoxylin and Eosin.. $MgSO_4$: magnesium sulfate.

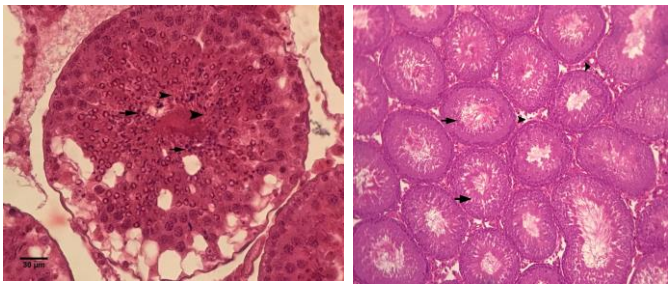

Figure 6. Testis section of left testis in the $MgSO_4$ (500 mg/kg) followed by I/R rats showing many normal seminiferous tubules (**arrow**) (H&E) (**Left**) with few spermatocyte **(Arrow head)** and testis section of right testis in the $MgSO_4$ (500 mg/kg) followed by I/R rats showing normal seminiferous tubules **(Arrow)** and interstitial cells **(Arrow head)** between tubules. H & E: Hematoxylin and Eosin. $MgSO_4$: magnesium sulfate.

Additionally, it has been revealed that semen oxidation acts via an increase in ROS levels and a decrease in antioxidant capacity (Hsieh et al., 2006), which leads to infertility (Masson and Brannigan, 2014). The low levels of ROS are critical for normal fertilization, capacitation, hyperactivation, and motility (Agarwal et al., 2009). Spermatozoa have high levels of polyunsaturated fatty acids, which are vulnerable to attacks by ROS (Ghalehkandi, 2018). Seminal plasma is endowed with MDA, SOD, and GPx (Ghalehkandi, 2018). During the oxidation, MDA levels are increased followed by decreases in SOD and GPx status (Hsieh et al., 2006). The ROS reacts with these enzymes via cell membrane lipid oxidative damage in sperm (Akhondi et al., 2013). Magnesium plays a prominent role in the human reproductive system, semen, and fertilization (Valsa et al., 2012). Moreover, seminal plasma has a critical role in the protection of sperms and acts as a buffer for sperm motility. Semen is composed of lipids, ions (e.e., citrate), calcium, Mg^{2+}, K^{+}, Na^{+}, zinc, chloride, proteins, and oxidative enzymes that protect the sperm from oxidative stress (Agarwal et al., 2013). Magnesium has been associated with oxidative enzymes and its depletion correlated with reduced antioxidant properties. It is reported that Mg^{2+} has an influence on SOD activity in retinal tissue (Korkmaz et al., 2013). Moreover, Mg^{2+} is required for GPx (Barbagallo et al., 2010). Oxidative stress is responsible for glaucoma pathogenesis and Mg^{2+}-induced oxidative enzyme levels can contribute to the progression of glaucoma (Korkmaz et al., 2013). Diminished cellular Mg^{2+} levels alter ATPase function and increase oxidative stress (Agarwal et al., 2013). Magnesium has neuroprotective activity in the brain and spinal cord ischemia (Yavuz et al., 2013). The injection of the Mg^{2+} reduced infarct size after I/R injury via decreasing generation and releasing free oxygen radicals in the left anterior descending artery (Ravn et al., 1999). The Mg^{2+} insufficiency might affect Na^{+} fluxes and elevated intracellular Na^{+}, which might lead to an increased Ca^{2+}. The severe ionic disturbances have been observed in I/R injury (Huang et al., 2014).

One possible mechanism of this effect is that Mg^{2+} may reduce endothelial and neuronal reperfusion injury by minimizing the Mg^{2+} use and lipid peroxidation. Furthermore, Mg^{2+} regulates ATP availability in the reperfusion phase (Yavuz et al., 2013). Eshraghi et al. (2015) reported that Mg^{2+} protected the liver against bile duct ligation-induced in rats. Based on their report, the protective activity of the Mg^{2+}mediates by diminishing MDA and increasing SOD and CAT activities (Eshraghi et al., 2015). The cellular Mg^{2+} has different functions in glycolysis, protein synthesis, respiration, and reproduction (Chandra et al., 2013). Testicular plasma and semen contain high levels of Mg^{2+} (Wong et al., 2001). The $MgSO_4$ treatment improved seminiferous tubules with many spermatocytes in the testes of experimental unilateral varicocele established rats (Asghari et al., 2018b). Magnesium isoglycyrrhizinate decreased MDA and increased SOD and GPx, which protected I/R-induced hepatic injury (Huang et al., 2014). It is reported that the number of spermatogonia A, preleptotene spermatocytes, mid-pachytene, spermatocytes, and spermatid was increased in Mg^{2+} treated animals. There have also been reports that revealed Mg^{2+} deficiency-induced morphological changes up to 40% in the spermatids (Chandra et al., 2013). Previous reports have mostly investigated the effects of Mg^{2+} on hepatic or cardiac I/R; moreover, they evaluated the hepatic enzymes, as well as inflammatory factors with limited information, existed on the ROS. Accordingly, there were no previous reports on the role of the Mg^{2+} in testicular I/R-injury to be compared with our results. In conclusion, new findings of the current study suggested that treatment with $MgSo_4$ had beneficial effects on I/R-induced rats. In addition, $MgSO_4$ improved seminiferous tubules with normal spermatocytes by increasing the oxidative defense system and decreasing ROS generation in I/R-induced rats. Further studies are required to determine the direct cellular and molecular actions of $MgSO_4$ against I/R injuries in rats and other species.

Ethics

We hereby declare all ethical standards have been respected in preparation of the submitted article.

Conflict of Interest

The authors declare that they have no conflict of interest.

Authors' Contribution

Study concept and design: Asghari, A., Abedi, G.
Acquisition of data: Moshkelani, S.
Analysis and interpretation of data: Asghari, A., Mortzavi, P.
Drafting of the manuscript: Asghari, A., Moshkelani, S.
Critical revision of the manuscript for important intellectual content: Asghari, A.
Statistical analysis: Jahandideh, A., Asghari, A.
Administrative, technical, and material support: Science and Research Branch Islamic Azad University, Tehran, Iran

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