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Magnesium sulfate protects testis against unilateral varicocele in rat

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Abstract

The aim of this study was to investigate the effect of magnesium sulfate ($MgSO_4$) on experimental unilateral varicocele-induced in rats. Sixty male Wistar rats were randomly divided into six experimental groups (n = 10). The Control group had no received any medications and surgery. The Sham group had no received any medication, abdominal cavity was opened without varicocele-induced. Varicocele group: abdominal cavity was opened, varicocele done without any medication treatment. In group 4 abdominal cavity was opened, varicocele-induced then animals orally treated with $MgSO_4$ (25 mg/kg) for 42 days. The groups 5 and 6 were similar to group 4, except animals received 50 and 100 mg/kg of $MgSO_4$, respectively. At the end of days 21 and 42, the abdomen was opened, the left testis extracted for histopathological studies. Also, from the cauda epididymis semen samples were collected to determine malondialdehyde, superoxide dismutase and glutathione peroxidase values. According to the results, there was a significant difference in testis damage grade in Varicocele group compared to the Control group ($P < 0.05$). The $MgSO_4$ significantly improved testis damage grade on day 42 ($P < 0.05$). The $MgSO_4$ treatment, dose dependently improved seminiferous tubules with many spermatocytes in the seminiferous tubules in experimental unilateral varicocele established rats after 21 and 42 days ($P < 0.05$). Also, administration of the $MgSO_4$ via a dose dependent manner significantly normalized malondialdehyde, superoxide dismutase and glutathione peroxidase values to the physiologic level in varicocele-induced rats after 21 and 42 days ($P < 0.05$). These results suggest administration of the $MgSO_4$ protects testis against unilateral varicocele in rat.

Keywords: magnesium sulfate, rat, testis, varicocele.

Introduction

One of the most important disorders in the male reproduction system is varicoceles. It happens because of pathological dilations of the venous pampiniform plexus of the spermatic cord and frequently appears in the left side because left testicular vein runs vertically and inserts into the left renal vein (Naughton *et al*. 2001; Sofikitis *et al*. 2014). Varicoceles are present in 35-40% of infertile men and represent a treatable form of infertility. The etiology of varicoceles is controversial and several factors are responsible for occurrence of varicoceles (Masson and Brannigan, 2014).

The relationship between varicocele with male infertility is known since ancient times where Celsius in the first century AD reported a link between dilated scrotal veins and testicular atrophy (Masson and Brannigan, 2014). Studies have documented reduced semen parameters in men with varicoceles. A relation exists between varicocele and semen oxidation where reactive oxygen species (ROS) levels increased and antioxidant capacity decreased in the semen of animal with varicocele (Hendin *et al*. 1999; Hsieh *et al*., 2006). These changes lead to abnormal sperm function and the infertility (Masson and Brannigan, 2014). So, the low levels of ROS are critical for normal fertilization, capacitation, hyperactivation and motility (Agarwal *et al*., 2009).

The ROS antioxidants such as superoxide dismutase (SOD), malondialdehyde (MDA) and glutathione peroxidase (GPx) have an essential effect in human reproduction. Polyunsaturated fatty acids (PUAFAs) are highly concentrated in spermatozoa and vulnerable to be attacked by ROS (Chi *et al*., 2008). For instance, infertile patients with varicocele have elevated levels of ROS, which known as pathophysiology of infertility (Masson and Brannigan, 2014).

Magnesium (Mg^{2+}) is one of such essential micronutrients and plays a key role within the cell in Mg-linked ATP processes, cell cycle, channel regulation, ATPase activity and metabolic regulation. Also, the intracellular Mg^{2+} levels interplay with other cell mediators such as nitric oxide (NO), calcium (Ca^{2+}) as well as cellular metabolism (Eshraghi *et al*., 2015). Seminal plasma Mg^{2+} is essential to mediate ADP-ribosylation and to inhibit endonuclease activity. The ADP-ribosylation involved in DNA repair in cells (Beltran-Parrazal *et al*., 2006). This Mg^{2+} protects chromosomal and extrachromosomal DNA from rapid degradation (Beltran-Parrazal *et al*., 2006). Also, Mg^{2+} protects livestock ram spermatozoa cells during freezing and thawing (Pesch *et al*., 2006). Recently, it is reported Mg^{2+} has a positive role in the control of ROS generation where administration of $MgSO_4$ decreased SOD in the bile duct ligation-induced liver injury in male Wistar rats (Eshraghi *et al*., 2015). Furthermore, Mg^{2+} deficiency impairs reproductive functions (Chandra *et al*., 2013). Also, total antioxidant status and Mg^{2+} levels of the seminal plasma are highly correlated with sperm motility and viability (Pesch *et al*., 2006).

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To the best of our knowledge, no report exists on the role of $MgSO_4$ on experimental unilateral varicocele in rat. So, our hypothesis was to investigate the possible role of $MgSO_4$ on testis histopathology as well as semen MDA, SOD and GPx in experimental unilateral varicocele-induced rat.

Materials and Methods

Animals

To survey possible effect of $MgSO_4$ on experimental unilateral varicocele-induced rat, 60 male Wistar rats (230-250 g) were purchased from Pasteur Institute (Tehran, Iran) and randomly allocated into six treatment groups (n = 10). The rats were housed individually in stainless steel wire-bottomed cages and resided under standard laboratory conditions, according to European community suggestions for laboratory animals at a temperature of 21 ± 2°C, relative humidity of 55-60% and a 12 h light period (starting at 8:00 AM). All animals had *ad libitum* access to chow pellets and fresh water. Animal were acclimatized to laboratory conditions for one week prior to experiments; each animal was used only once and killed immediately after the experiment. All experimental procedures were carried in accordance with the Guide for the Care and Use of Laboratory Animals to Investigate Experimental Pain in Animals (Zimmermann, 1983). Animal handling and experimental procedures were performed according to the Guide for the Care and Use of Laboratory Animals by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and the current laws of the Iranian government. All protocols for animal experiments were approved by the institutional animal Ethical Committee, Islamic Azad University, Science and Research Branch, Tehran, Iran.

Chemicals

$MgSO_4$ from Sigma Chemicals (Poole, Dorset, UK) and assay kits of MDA, SOD and GPx (Randox Laboratories Ltd., Crumlin, Antrim, United Kingdom) were purchased.

Experimental creation of varicocele

All surgical procedures were performed under anesthesia by intraperitoneal (i.p.) injection of 60 mg/kg of ketamine hydrochloride 10% and 10 mg/kg of xylazine hydrochloride 2%. Then experimental varicocele was created (Turner, 2001; Sahin *et al*., 2005). The upper left abdominal quadrant was approached through a midline laparotomy incision. Then to reveal the left kidney, the abdominal contents were packed to the right side. Herein, the renal and adrenal veins and the left spermatic vein, inserts into the left renal vein. Surrounding fat and connective tissues of the left renal vein cleared to the insertion of the spermatic and adrenal veins. With a midline incision in the left renal vain was exposed and after fine dissection of proximal left renal vein, left renal vein was tied using a silk suture (4-0). At the point of medial to insertion of the adrenal and spermatic vain into the renal, a metal probe (diameter ranging from 0.4-0.85 based on size of renal vein) was placed. The ligature was made around the probe, then probe removed and the vain allowed expanding within the boundary of ligature. This procedure leads to decrease renal vain diameter to one half. The midline incision of the abdominal wall and the anterior abdominal muscles were repaired, separately (Celik-Ozenci *et al*. 2006).

Experimental procedure

Sixty male Wistar rats were randomly divided into 6 experimental groups (n = 10). The Control group had no received any medication and surgery. The Sham group had no received any medication, abdominal cavity was opened without varicocele establishment. Varicocele group: abdominal cavity was opened, varicocele-induced without further medication therapy. In group 4, abdominal cavity was opened, varicocele-induced and animal orally received $MgSO_4$ (diluted in water and orally administrated, 25 mg/kg) as medication, once a day for 42 days. The groups 5 and 6 were similar to group 4, except animals received 50 and 100 mg/kg of $MgSO_4$, respectively. Animals in the other groups (Control and Sham), received the equal volume of distilled water once a day for a similar period. After the 21 and 42 days post $MgSO_4$ treatment, rats fasted and euthanized with an overdose injection of pentobarbital (300 mg/kg, i.p.). Peritoneum on each animal was opened by an incision, testes were taken out for histopathologic investigations and epididymal fluid MDA, SOD and GPx levels.

Tissue processing

The fixation of the testis is started in the live anesthetized animal by injecting 3% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.2 under the albuginea; within a few minutes this causes sufficient hardening of the testis tissue to enable one to cut neat slices from it with a razor blade (Dal Lago and Lucke, 1973). Then, testis tissue samples from the experimental rats were fixed at BOUIN's solution for complete fixation and processed for paraffin sectioning. A tissue section about 5 µm thickness were taken and stained with hematoxylin and eosin (H & E). The testis sections were graded numerically to assess the degree of histological changes associated with seminiferous tubule injury as previously described by Johnsen (1970) as bellow:

- 10: complete spermatogenesis and perfect tubules;
- 9 many spermatozoa present but disorganized spermatogenesis;
- 8: only a few spermatozoa present;
- 7: no spermatozoa but many spermatids present;
- 6: only a few spermatids present;
- 5: no spermatozoa or spermatids present but many spermatocytes present;
- 4 :only a few spermatocytes present;
- 3 :only spermatogonia present;
- 2: no germ cells present;
- 1: neither germ cells nor Sertoli cells present.

Measurement of SOD, MDA and GPx

At the end of 21 and 42 days, Semen samples were collected from the Caudal epididymis and homogenized in 10% (W/V) ice-cold buffer (0.1 M phosphate buffer, pH 7.4 + 150 mM KCl). The homogenate was centrifuged at 9000 rpm for 20 min to obtain a supernatant which was used for SOD, MDA and GPx estimations (Sharma *et al*., 2012). The role of SOD is to accelerate the dismutation of the toxic superoxide radical (O_2), produced during the oxidative energy processes, to hydrogen peroxide and molecular oxygen. This method employs xanthine and xanthine oxidase (XOD) to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (I.N.T.) to form a red formazan dye detectable at 505 nm (Woolliams *et al*., 1983). Malondialdehyde is a standard to determine free radical damage. MDA was formed as an end product of lipid peroxidation and treated with thiobarbituric acid (TBA) to produce a colored product that was measured at 532 nm (Placer *et al*., 1966). GPx catalyses the oxidation of glutathione and in the presence of glutathione reductase and NADPH, oxide glutathione converts to the reduced form by changes in oxidation of NADPH to $NADP^+$. The GPx level was measured in absorbance of 340 nm (Paglia and Valentine, 1967).

Statistical analysis

Data were prepared in excel, the parametric data analyzed with one way analysis of variance (ANOVA) using SPSS 16.0 for Windows (SPSS, Inc., Chicago, IL, USA). Data were expressed as mean values ± standard error of mean (SEM). Where heterogenecity occurred, the groups were separated using Duncan Multiple Range Test (Duncan, 1957). P values of <0.05 were considered to denote significant differences between groups.

Results

Result of score of histological changes with seminiferous tubules injury is presented in Fig. 1. Also, the effect of $MgSO_4$ on testis histopathology after 21 and 42 days are presented in Fig. 2 to 11. The effect of $MgSO_4$ on semen MDA, SOD and GPx on days 21 and 42 post experimental unilateral varicocele rat is shown in Table 1.

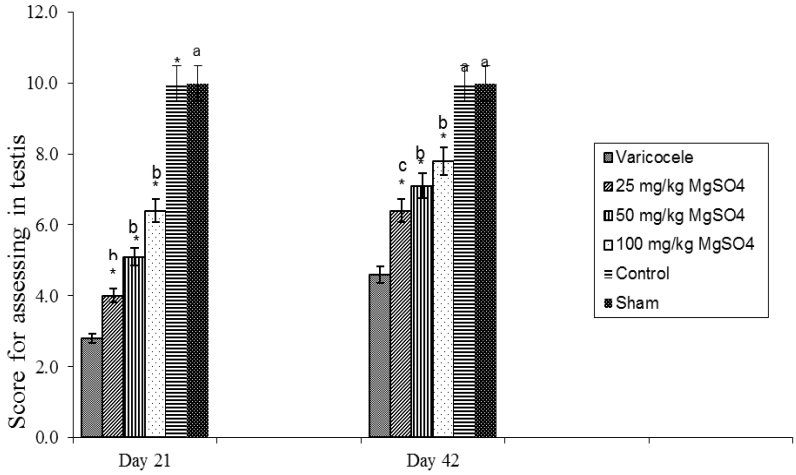

Figure 1. Score of histological changes associated with seminiferous tubules injury in unilateral varicocele-induced rat. Asterisks indicate significant difference on testis damage score between groups compared to Varicocele group ($P < 0.05$). Different letters (^{a,b,c}) indicate significant differences between treatments ($P < 0.05$).

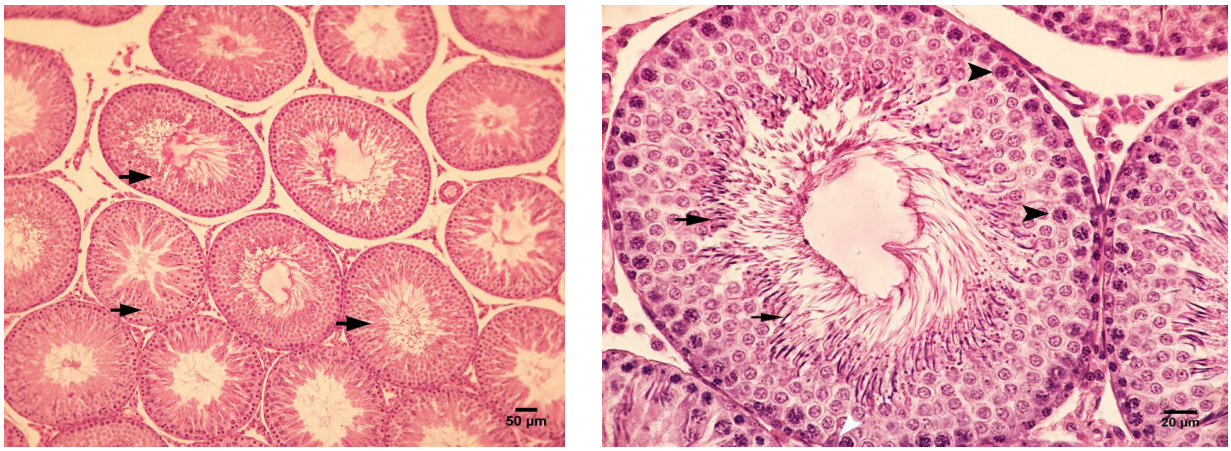

Figure 2. Testis section of control rats showing normal seminiferous tubules (arrow; H&E; Left) and normal spermatogenesis with spermatocytes (Black arrowhead), Sertoli (white arrow) and spermatozoa (black arrow; H & E; Right). H & E: hematoxylin and eosin.

Table 1. Effect of different levels of magnesium sulfate on semen values of Malondialdehyde, Superoxide dismutase and Glutathione peroxidase in experimental unilateral varicocele-induced rat after 21 and 42 days.

Group	MDA (nmol/ml)		SOD (IU)		GPx (IU)	
	21 day	42 day	21 day	42 day	21 day	42 day
Control	198.2 ± 10.2^{c}	210.1 ± 9.89^{c}	179.08 ± 8.65^{a}	191.11 ± 10.28^{a}	7161.23 ± 40.56^{a}	7120.10 ± 48.01^{a}
Sham	195.57 ± 13.15^{c}	204.15 ± 12.26^{c}	180.53 ± 10.78^{a}	188.36 ± 46.72^{a}	7214.26 ± 44.83^{a}	7116.13 ± 39.14^{c}
Varicocele	361.22 ± 28.26^{a}	387.41 ± 30.12^{a}	89.09 ± 9.04^{c}	88.14 ± 5.37^{c}	5431.27 ± 39.21^{c}	5250.17 ± 50.17^{c}
$MgSO_4$ (25 mg/kg)	360.04 ± 27.59^{a}	269.2 ± 21.02^{b}	92.24 ± 8.75^{c}	120 ± 14.97^{c}	5650.7 ± 40.16^{c}	6280.19 ± 49.34^{c}
$MgSO_4$ (50 mg/kg)	279.42 ± 25.07^{b}	259.82 ± 20.11^{b}	130.45 ± 12.80^{ab}	$165.8 \pm .04^{b}$	6132.2 ± 43.64^{b}	6716.31 ± 50.43^{b}
$MgSO_4$ (100 mg/kg)	209.17 ± 15.22^{c}	210.16 ± 11.20^{c}	166.45 ± 14.13^{a}	188.24 ± 18.17^{ab}	6895.35 ± 44.07^{a}	6897.24 ± 51.67^{a}

MDA: malondialdehyde. SOD: superoxide dismutase. GPx: glutathione peroxidase. There are significant differences between groups with different superscripts in a column ($P < 0.05$).

As seen, varicocele group had the lowest testis damage grade compared to the other groups ($P < 0.05$). Also, the testis damage grade was higher among the Control and Sham groups but not significant ($P < 0.05$). Asterisks shown a significant difference on testis damage grade in Control, Sham and $MgSO_4$ treated groups compared to Varicocele group ($P < 0.05$). There was no significant difference on testis damage score in rat treated with different levels of $MgSO_4$ on day 21 ($P < 0.05$) while in a dose dependent manner it was improved on day 42 ($P < 0.05$).

The effect of $MgSO_4$ on testis histopathology after 21 and 42 days is shown in Fig. 2 to 11. According to the results, testis section of control rats had shown normal seminiferous tubules and spermatogenesis with spermatocytes, Sertoli and spermatozoa on days 21 and 42 (Fig. 2). To reduce duplication in figures, we presented just one figure for the Control group.

As observed in Fig. 3, normal seminiferous tubules with interstitial cells, spermatogenesis with spermatogonia, Sertoli and spermatozoa were detected in testis cross sections of sham group entire the study (days 21 and 42). Herein, we provide a figure to minimize the duplication.

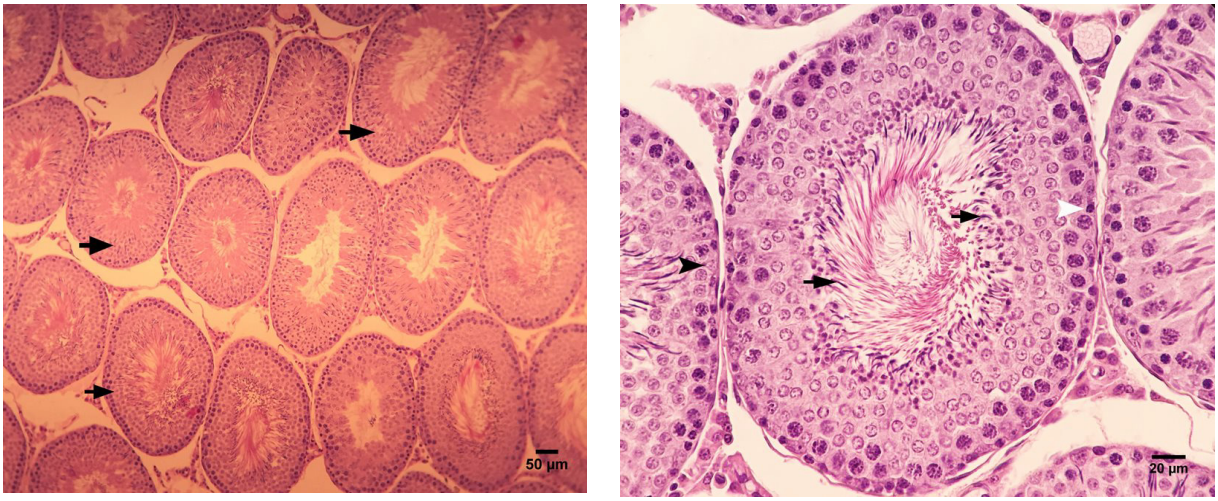

Figure 3. Testis section of shame rats showing normal seminiferous tubules (arrow) with interstitial cells (arrowhead; H & E; Left) and normal spermatogenesis with spermatogonia (white arrowhead), Sertoli (black arrowhead) and spermatozoa (arrow; H & E; Right). H & E: hematoxylin and eosin.

Based on the results of Fig. 4, seminiferous tubules degenerated and loss of spermatogenesis with few spermatocytes was observed in degenerated testis tubules of unilateral varicocele-induced rat on day 21.

In this study, large degree of degeneration occurred in the seminiferous tubules and loss in spermatogenesis with few spermatocytes in unilateral varicocele on day 42 (Fig. 5).

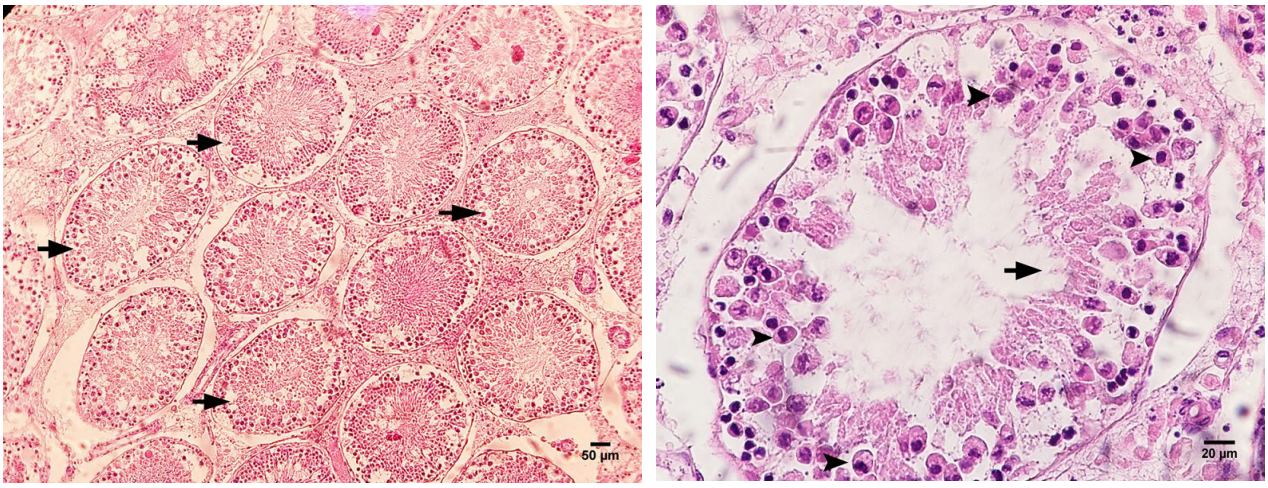

Figure 4. Testis section of unilateral varicocele rats (21 day) showing degenerated seminiferous tubules (arrow) and loss of spermatogenesis (H & E; Left) and degenerated seminiferous tubules (arrow) with few spermatocyte (arrowhead) in degenerated tubules (21 day; H & E; Right). H & E: hematoxylin and eosin.

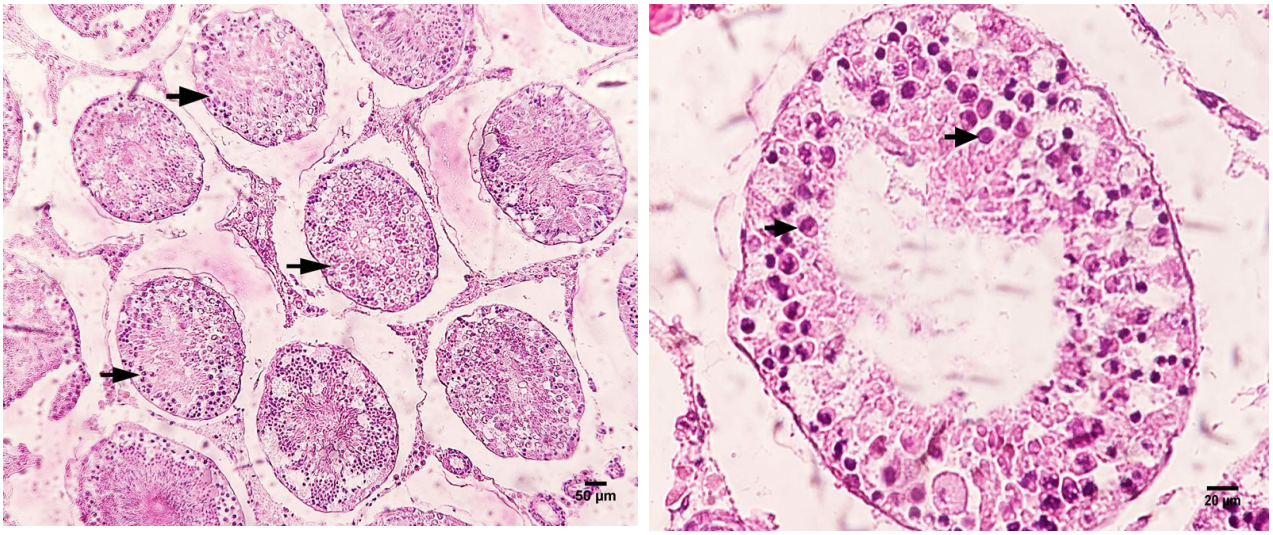

Figure 5. Testis section of unilateral varicocele rats (42 day) showing degenerated seminiferous tubules (arrow) and loss of spermatogenesis (H & E; Left) and degenerated seminiferous tubules with few spermatocyte (arrow) in degenerated tubules (42 day; H & E; Right). H & E: hematoxylin and eosin.

As shown in Fig. 6, administration of 25 mg/kg of $MgSO_4$ had a scarce effect on seminiferous tubules (Fig. 6-left picture) and spermatocyte (Fig. 6-right picture) on day 21 in experimental unilateral varicocele-induced rat.

Administration of $MgSO_4$ (25 mg/kg) improved testis characteristics with few normal seminiferous tubules and spermatocyte in seminiferous tubules of experimental unilateral varicocele-induced rat after 42 days (Fig. 7).

Herein, many normal seminiferous tubules and many spermatocyte in the seminiferous tubules were observed in unilateral varicocele rats treated with 50 mg/kg of $MgSO_4$ for 21 days (Fig. 8) and 42 days (Fig. 9).

According to the Fig. 10 and 11, administration of $MgSO_4$ (100 mg/kg) leads to many normal seminiferous tubules with spermatocyte in the seminiferous tubules in experimental unilateral varicocele rats after 21 and 42 days (Fig. 10 and 11, respectively).

The effect of different levels of $MgSO_4$ on semen values of MDA, SOD and GPx in experimental unilateral varicocele-induced rat is shown in Table 1. According to the results, MAD level significantly increased in Varicocele group compared to Control group on days 21 and 42 ($P < 0.05$). Administration of different levels of $MgSO_4$ (25, 50 and 100 mg/kg) significantly lessened the elevated MDA level after 21 and 42 days in varicocele-induced rats compared to the Control group ($P < 0.05$).

Also, a significant decrease detected on semen SOD and GPx levels in varicocele rats ($P < 0.05$) while, administration of $MgSO_4$ in a dose dependent manner improved semen SOD and GPx levels ($P < 0.05$).

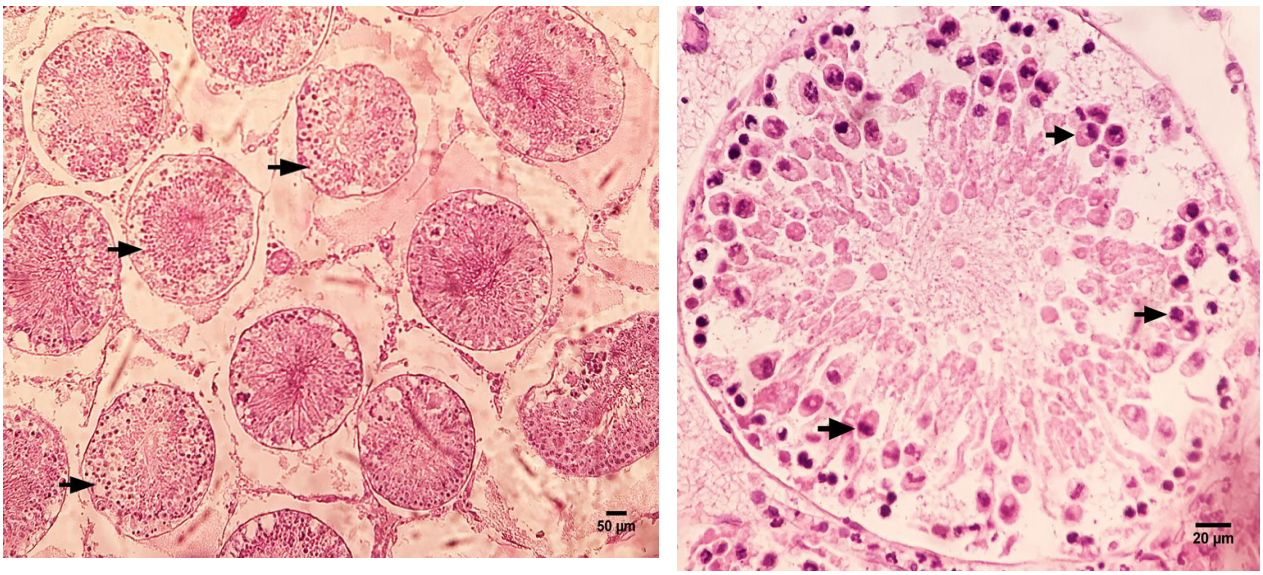

Figure 6. Testis section of unilateral varicocele-induced rats treated by 25 mg/kg of $MgSO_4$ (21 day) showing degenerated seminiferous tubules (arrow; H & E; Left) and degenerated seminiferous tubules with few spermatocyte (arrow) in degenerated tubules (21 day; H & E; Right). H & E: hematoxylin and eosin. $MgSO_4$: magnesium sulfate.

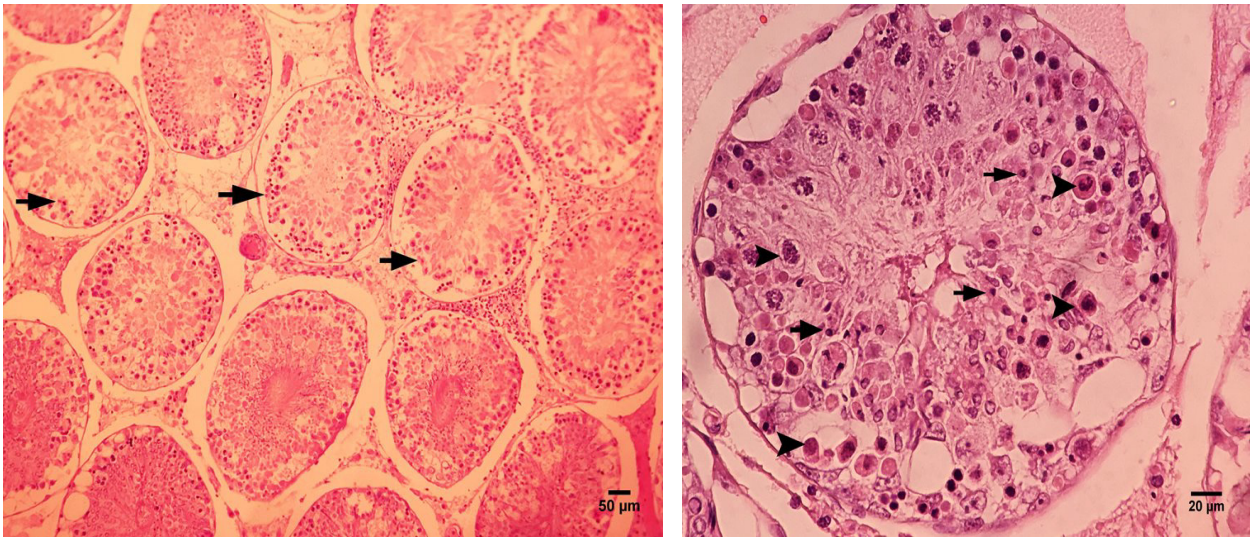

Figure 7. Testis section of unilateral varicocele-induced rats treated by 25 mg/kg of $MgSO_4$ (42 day) showing few normal seminiferous tubules (arrow) and many degenerated tubules (H & E; Left) and many spermatocyte (arrowhead) and few spermatid in seminiferous tubules (42 day; H & E; Right). H & E: hematoxylin and eosin. $MgSO_4$: magnesium sulfate.

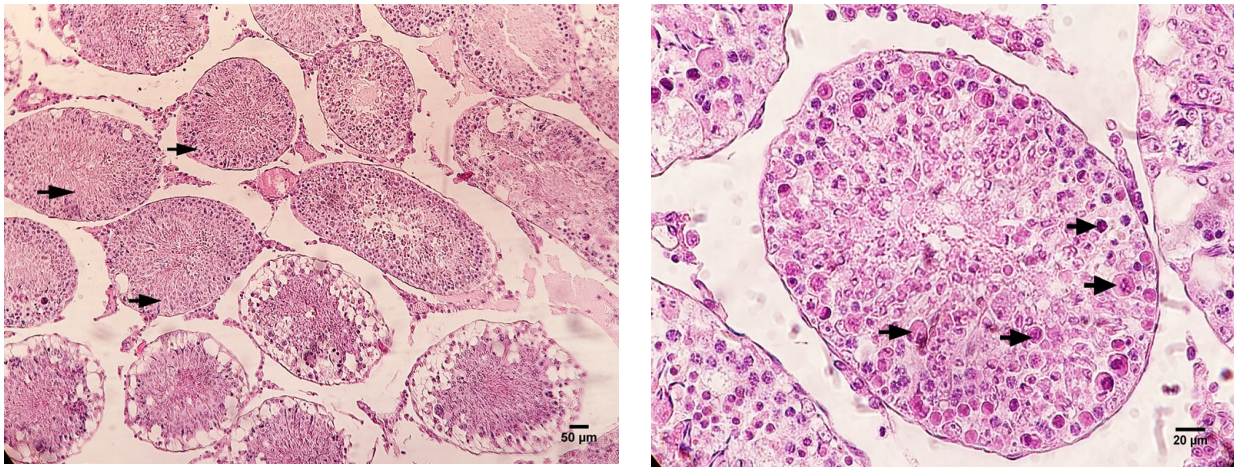

Figure 8. Testis section of unilateral varicocele-induced rats treated by 50 mg/kg of $MgSO_4$ (day 21) showing many normal seminiferous tubules (arrow; H & E; Left) and many spermatocyte (arrow) in seminiferous tubules (day 21; H & E; Right). H & E: hematoxylin and eosin. $MgSO_4$: magnesium sulfate.

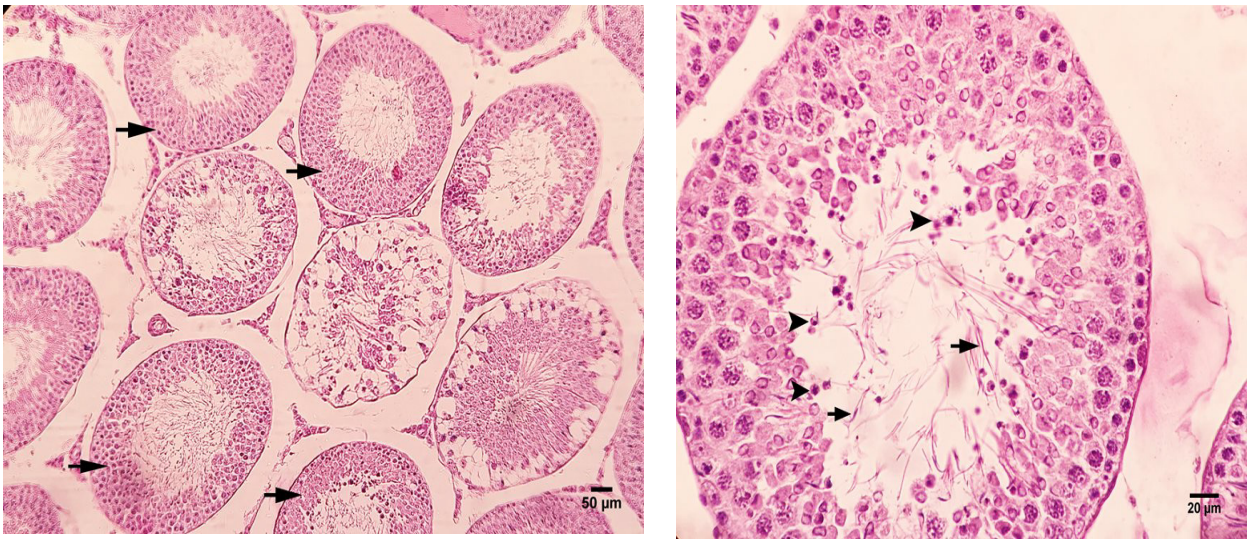

Figure 9. Testis section of unilateral varicocele-induced rats treated by 50 mg/kg of $MgSO_4$ (day 42) showing many normal seminiferous tubules (arrow; H & E; Left) and many spermatids (arrowhead) and few spermatozoa (arrow) in seminiferous tubules (day 42; H & E; Right). H & E: hematoxylin and eosin. $MgSO_4$: magnesium sulfate.

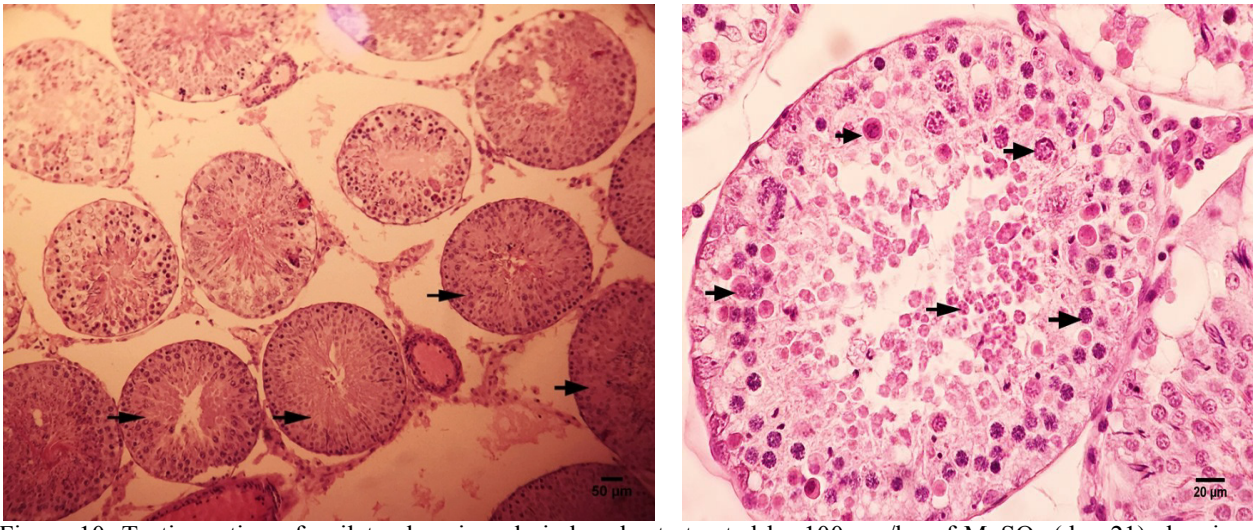

Figure 10. Testis section of unilateral varicocele-induced rats treated by 100 mg/kg of $MgSO_4$ (day 21) showing many normal seminiferous tubules (arrow; H & E; Left) and many spermatocyte (arrow) in seminiferous tubules (day 21; H & E; Right). H & E: hematoxylin and eosin. $MgSO_4$: magnesium sulfate.

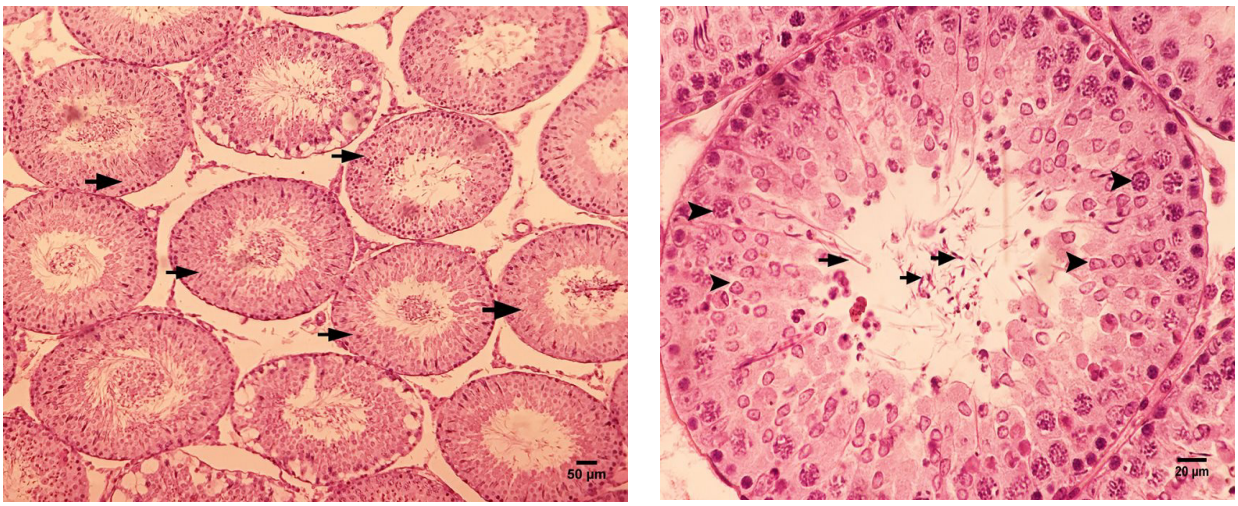

Figure 11. Testis section of unilateral varicocele-induced rats treated by 100 mg/kg of $MgSO_4$ (day 42) showing normal seminiferous tubules (arrow; H & E; Left) and many spermatocyte (arrowhead) and few spermatozoa (arrow) in seminiferous tubules (H & E; Right). H & E: hematoxylin and eosin. $MgSO_4$: magnesium sulfate.

Discussion

To the best of our knowledge, there are limited studies describing the role of $MgSO_4$ on spermatozoa characteristics in experimental varicocele in rat. This study was conducted for the first time to investigate the effect of $MgSO_4$ on testis histopathology and semen MDA, SOD and GPx in experimental unilateral varicocele-induced rat. As seen from the results, varicocele leads to degeneration of seminiferous tubules and loss in spermatogenesis and spermatocytes. However, administration of $MgSO_4$, dose dependently improved semen oxidative enzyme defense system in experimental unilateral varicocele-induced rat after 21 and 42 days.

Animal models of varicocele have been developed in several species, including the rat to determine the pathophysiology of varicocele. Surgical varicocele involves partial obstruction of the left renal vein, causing a varicosity of the left spermatic vein (Koksal *et al*., 2002). However, the mechanisms responsible for impaired spermatogenesis in animals with iatrogenically induced varicoceles remains unclear. It is well documented impairment in the testicular blood flow has adverse effect on testicular dysfunction (Li *et al*., 1999). It is hypothesized varicoceles induce their noxious effect through elevating the scrotal temperature. The elevated intrascrotal temperature leads to reduction in testosterone synthesis by Leydig cells and reduced Sertoli cell secretory function (Sofikitis *et al*. 2014).

Sertoli cells play a crucial role in the spermatogenesis. These cells produce different proteins that are needed for the Sertoli-germ cell interactions (Li *et al*., 1999). As observed in this study, varicocele destroyed seminiferous tubules with minute spermatogenesis and spermatocyte. Unilateral varicocele on the left side is the much more frequent because left testicular vein runs vertically and inserts into the left renal vein (Sofikitis *et al*., 2014). Because of that in this study, we focused on effect of left testicular varicocele than to the right side.

A positive correlation reported with varicocele

and testicular defense system where varicocele increased ROS and decreased antioxidant capacity levels in the semen. In this regard, ROS and interleukin levels elevated and total antioxidant capacity diminished in infertile patients with varicocele (Nallella *et al*., 2004; French *et al*., 2008). Seminal plasma is endowed with frequent enzymatic antioxidants includes SOD and GPx (Ghiasi Ghalehkandi *et al*., 2015). ROS include hydrogen peroxide and unstable free radicals with unpaired electrons in their outer orbits. They produce from mitochondria and plasma membranes of abnormal spermatozoa. Excess ROS generation or decreased antioxidant defenses in the seminal plasma damages spermatozoa via oxidative stress. So, low levels of ROS are critical for normal spermatogenesis and fertility (Agarwal *et al*., 2009).

Sperm membranes contain large amounts of polyunsaturated fatty acids (PUFAs) in spermatozoa which are vulnerable to be attacked by ROS (Hwang *et al*., 2014). MDA is the end product of lipid peroxidation, where the elevation in testicle MDA levels is a sign of lipid peroxidation and leads to infertility (Hsieh *et al*., 2006). As observed in this study, the MDA level significantly elevated in varicocele-induced rat compared to Control group. MDA levels increased in the semen of patients with varicocele (Koksal *et al*., 2003; Cervellione *et al*., 2006) so, increased MDA levels is associated with higher grades of varicocele (Koksal *et al*., 2002).

On the basis of the data obtained, varicocele decreased SOD and GPx levels in experimental unilateral varicocele-induced rat. Also, 25 and 50 mg/kg of $MgSO_4$ were not able to normalize the SOD and GPx levels while the level of 100 mg/kg increased the enzymes to the normal level. SOD is a fundamental part of the cellular antioxidant defense system (Ghiasi Ghalehkandi, 2015). It is the first defense line against oxidative stress with dismutation of superoxide anion radicals to H_2O_2 (Asadpour *et al*., 2013). Serum SOD levels increased in $CrCl_3$- (Ghiasi Ghalehkandi, 2015) and Cadmium-exposed rat (Suru, 2008). GPx is an enzyme family with peroxidase activity, impresses its role by protecting sperm against peroxidative damage (Hsieh *et al*., 2006). A correlation exists among GPx levels and asthenozoospermia (Ghiasi Ghalehkandi, 2015).

Testicular plasma and semen contains high concentrations of minerals and ions such as Ca^{2+}, Zn, Cu and Mg^{2+} (Wong *et al*., 2001). In this study, $MgSO_4$ treatment, dose dependently improved seminiferous tubules with many spermatocytes in the seminiferous tubules in experimental unilateral varicocele established rats. Also, $MgSO_4$ normalized the MDA, SOD and GPx values in varicocele-induced rats. It is revealed, Mg^{2+} supplement has considerable benefit in patients exposed to Cisplatin (Willox *et al*., 1986). Also, Eghbali *et al*. (2010) reported seminal Ca^{2+} and Mg^{2+} associated with semen characteristics and have effect on motility and viability of the spermatozoa. Seminal total antioxidant capacity is correlated with seminal plasma Mg^{2+} content. So, decreased level of Mg^{2+} reduces the oxidative defense system of the semen (Eghbali *et al*., 2010).

Mg^{2+} play key role in the control of ROS generation where administration of $MgSO_4$ decreased SOD in bile duct ligation-induced liver injury in male Wistar rats (Eshraghi *et al*., 2015). Moreover, $MgSO_4$ protects hepatic tissue against Vanadium-induced lipid peroxidation in rat (Scibior *et al*., 2013). Mg^{2+} is associated with oxidative stress in different pathologic conditions such as diabetes, hypertension and atherosclerosis (Wolf *et al*., 2009). The direct molecular mechanism(s) for how Mg^{2+} acts as antioxidant agent is not fully elicited. Perhaps, Mg^{2+} complexes with phospholipids; reduce fluidity and permeability of the membrane (Eshraghi *et al*., 2015). The Mg^{2+} dependent inhibition of cell growth might be due to ROS-mediated DNA damage. Perhaps, Mg^{2+} availability concurrent mechanisms of cell damage by a primary or ancillary oxidative stress (Wolf *et al*., 2009).

In conclusion, an important new finding of the study is that the administration of $MgSO_4$ improved seminiferous tubules with normal spermatocytes by increase testis oxidative defense system via decrease in ROS generation in varicocele-induced rats. Actually, there was no similar research to compare our results on mediatory role of $MgSO_4$ on experimental unilateral varicocele-induced rat. We think further researches needs to determine direct cellular and molecular action of $MgSO_4$ against varicocele.

References

Agarwal A, Sharma RK, Desai NR, Prabakaran S, Tavares A, Sabanegh E. 2009. Role of oxidative stress in pathogenesis of varicocele and infertility. *Urology,* 73:461-469.

Asadpour R, Azari M, Hejazi M, Tayefi H, Zaboli N. 2013. Protective effects of garlic aquous extract (*Allium sativum*), vitamin E, and N-acetylcysteine on reproductive quality of male rats exposed to lead. *Vet Res Forum,* 4:251-257.

Beltran-Parrazal L, López-Valdés HE, Brennan KC, Díaz-Muñoz M, de Vellis J, Charles AC. 2006. Mitochondrial transport in process of cortical neurons is independent of intracellular calcium. *Am J Physiol Cell Physiol*, 291:C1193-C1197.

Celik-Ozenci C, Bayram Z, Akkoyunlu G, Korgun ET, Erdogru T, Seval Y, Ustunel I, Baykara M, Demir R. 2006. Localization of NGF and nNOS in varicocele-induced rat testis. *Acta Histochem,* 107:435-442.

Cervellione RM, Cervato G, Zampieri N, Corroppolo M, Camoglio F, Cestaro B, Ottolenghi A. 2006. Effect of varicocelectomy on the plasma oxidative stress parameters. *J Pediatr Surg,* 41:403-406.

Chandra AK, Sengupta P, Goswami H, Sarkar M. 2013. Effects of dietary magnesium on testicular histology, steroidogenesis, spermatogenesis and oxidative stress markers in adult rats. *Indian J Exp Biol,* 51:37-47.

Chi HJ, Kim JH, Ryu CS, Lee JY, Park JS, Chung DY, Choi SY, Kim MH, Chun EK, Roh SI. 2008. Protective effect of antioxidant supplementation in sperm-preparation medium against oxidative stress in human spermatozoa. *Hum Reprod,* 23:1023-1028.

Dal Lago A, Lucke S. 1973. A method of fixing rat

testis for light and electron microscopy. *Stain Technol,* 48:289-295.

Duncan BD. 1957. Multiple range tests for correlated and heteroscedastic means. *Biometrics*, 13:359-364.

Eghbali M, Alavi-Shoushtari SM, Asri-Rezaei S, Khadem Ansari MH. 2010. Calcium, magnesium and total antioxidant capacity (TAC) in seminal plasma of water buffalo (*Bubalus bubalis*) bulls and their relationships with semen characteristics. *Vet Res Forum,* 1:12-20.

Eshraghi T, Eidi A, Mortazavi P, Asghari A, Tavangar SM. 2015. Magnesium protects against bile duct ligation-induced liver injury in male Wistar rats. *Magnes Res*, 28:32-45.

French DB, Desai NR, Agarwal A. 2008. Varicocele repair: does it still have a role in infertility treatment? *Curr Opin Obstet Gynecol*, 20:269-274.

Ghiasi Ghalehkandi J. 2015. Garlic (*Allium sativum*) juice protects from semen oxidative stress in male rats exposed to chromium chloride. *Anim Reprod*, 11:526-532.

Ghiasi Ghalehkandi J, Hassanpour S, Issabeagloo E, Asghari A. 2015. Assessment of the effects of red onion (*Allium cepa Linn*) juice on semen oxidative status compared to Zn sulfate in rats. *Anim Reprod*, 12:298-304.

Hadwan MH, Almashhedy LA, Alsalman ARS. 2014. Study of the effects of oral zinc supplementation on peroxynitrite levels, arginase activity and NO synthase activity in seminal plasma of Iraqi asthenospermic patients. *Reprod Biol Endocrinol,* 12:1. doi: 10.1186/1477-7827-12-1.

Hendin BN, Kolettis PN, Sharma RK, Thomas Jr AJ, Agarwal A. 1999. Varico- cele is associated with elevated spermatozoal reactive oxygen species production and diminished seminal plasma antioxidant capacity. *J Urol,* 161:1831-1834.

Hsieh Y, Chang C, Lin C. 2006. Seminal malondialdehyde concentration but not glutathione peroxidase activity is negatively correlated with seminal concentration and motility. *Int J Biol Sci,* 2:23-29.

Johnsen S. 1970. Testicular biopsy score count–a method for registration of spermatogenesis in human testes: normal values and results in 335 hypogonadal males. *Hormones*, 1:2-25.

Koksal IT, Usta M, Orhan I, Abbasoglu S, Kadioglu A. 2003. Potential role of reactive oxygen species on testicular pathology associated with infertility. *Asian J Androl,* 5:95-99.

Koksal T, Erdogru T, Toptas B, Gulkesen KH, Usta M, Baykal A, Baykara M. 2002. Effect of experimental varicocele in rats on testicular oxidative stress status. *Andrologia,* 34:242-247.

Li H, Dubocq F, Jiang Y, Tiguert R, Gheiler EL, Dhabuwala CB. 1999. Effect of surgically induced varicocele on testicular blood flow and sertoli cell function. *Urology,* 53:1258-1262.

Masson P, Brannigan RE. 2014. The varicocele. *Urol Clin N Am,* 41:129-144.

Nallella KP, Allamaneni SS, Pasqualotto FF, Sharma RK, Thomas Jr AJ, Agarwal A. 2004. Relationship of interleukin-6 with semen characteristics and oxidative stress in patients with varicocele. *Urology,* 64:1010-1013.

Naughton CK, Nangia AK, Agarwal A. 2001. Varicocele and male infertility: Part II. Pathophysiology of varicoceles in male infertility. *Hum Reprod Update,* 7:473-481.

Paglia DE, Valentine VN. 1967. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med,* 70:158-169.

Pesch S, Bergmann M, Bostedt H. 2006. Determination of some enzymes and macro-and microelements in stallion seminal plasma and their correlations to semen quality. *Theriogenology,* 66:307-313.

Placer ZA, Cushman LL, Johnson BC. 1966. Estimation of product of lipid peroxidation (malondialdehyde) in bio-chemical systems. *Anal Biochem,* 16:359-364.

Sahin Z, Bayram Z, Celik-Ozenci C, Akkoyunlu G, Seval Y, Erdogru T, Ustunel I, Baykara M, Demir R. 2005. Effect of experimental varicocele on the expressions of notch 1, 2, 3 in rat testes: an immunohistochemical study. *Fertil Steril,* 83:86-94.

Scibior A, Golwbiowska D, Niedzwiecka I. 2013. Magnesium can protect against vanadium-induced lipid peroxidation in the hepatic tissue. *Oxid Med Cell Longev*, 2013:802734. doi: doi:10.1155/2013/802734.

Sharma P, Huq AU, Singh R. 2012. Cypermethrin induced reproductive toxicity in male Wistar rat: protective role of *Tribulus terrestris*. *J Environm Biol,* 34:857-862.

Sofikitis N, Stavrou S, Skouros S, Dimitriadis F, Tsounapi P, Takenaka A. 2014. Mysteries, facts, and fiction in varicocele pathophysiology and treatment. *Eur Urol Suppl,* 13:89-99.

Suru SM. 2008. Onion and garlic extracts lessen cadmium-induced nephrotoxicity in rats. *Biometals,* 21:623-633.

Turner TT. 2001. The study of varicocele through the use of animal models. *Hum Reprod Update,* 7:78-84.

Willox JC, McAllister EJ, Sangster G, Kaye SB. 1986. Effects of magnesium supplementation in testicular cancer patients receiving cis-platin: a randomised trial. *Br J Cancer,* 54:19-23.

Wolf FI, Trapani V, Simonacci M, Boninsegna A. 2009. Magnesium deficiency affects mammary epithelial cell proliferation: involvement of oxidative stress. *Nutr Cancer*, 61:131-136.

Wong WY, Flik G, Groenen PMW, Swinkels DW, Thomas CMG, Copius-Peereboom JHJ, Merkus HMWM, Steegers-Theunissen RPM. 2001. The impact of calcium, magnesium, zinc, and copper in blood and seminal plasma on semen parameters in men. *Reprod Toxicol,* 15:131-136.

Woolliams JA, Wiener G, Anderson PH, McMurray CH. 1983. Improved method for the determination of blood glutathione. *Res Vet Sci*, 34:253-256.

Zimmermann M. 1983. Ethical guidelines for investigations of experimental pain in conscious animals. *Pain,* 16:109-110.