Original Article

Do Exercise and Magnesium Sulfate Improve Infertility Caused by Lithium Carbonate in Male Rats?

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Abstract

Background: Lithium (Li) is widely used in the treatment of bipolar disorder, but it may lead to toxicity in the reproductive system. Considering the harmful effect of Li consumption on fertility and the positive effect of magnesium sulfate ($MgSo_4$) and moderate-intensity training (MIT) on improving the quality of men's sperm, the current research was conducted to determine the impact of MIT and $MgSo_4$ on infertility caused by Li.

Materials and Methods: Seventy-two male rats were divided into 12 groups, control, Li10 mg/kg/day/ip, $MgSo_4$ 80 mg/kg/day/ip; MIT; Li40 mg/kg/day/ip; Li10+$MgSo_4$; Li10+MIT; Li10+$MgSo_4$+MIT; Li40+$MgSo_4$; Li40+MIT; Li40+$MgSo_4$+MIT. All animals received the drugs every day. The groups under the exercise protocol followed this program for 42 days (6 weeks). Total sperm count, sperm concentration, total motility, and progressive motility were analyzed. A blood sample was taken from the heart to quantify testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH).

Results: Li40 mg/kg decreased the progressive motility and quantity of sperms together with nonprogressive motility and immobile sperms increased significantly. Administration of $MgSo_4$ and MIT alone and simultaneously led to a significant improvement in the above mentioned parameters. Li40 mg/kg reduced the serum level of testosterone and LH compared to the control group. On the other hand, the administration of $MgSo_4$ and MIT together with Li40 (Li40+$MgSo_4$+MIT) did not have any effect on serum testosterone levels.

Conclusions: Li probably causes damage to reproductive functions by affecting the antioxidant system. However, $MgSo_4$ and MIT reduce the impacts of Li on the reproductive system and improved its performance.

Keywords: Infertility, lithium, $MgSo_4$, moderate-intensity training

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Introduction

Lithium (Li) has been a valuable first-line antimanic agent widely used in the treatment of bipolar disorder for half a century.[1] Li reduces the risk of suicide in the mentioned patients and has a positive effect on the treatment of migraine and cluster headaches, arthritis, leukopenia, and neurological diseases. Despite prominent therapeutic effects in bipolar disorder, long-term exposure to Li may lead to toxicity in various systems and complications including renal failure, diabetes insipidus, hypothyroidism, goiter development, and brain damage.[2,3] In addition, infertility and decreased libido are the other adverse effects of Li. Several reproductive

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abnormalities in association with Li usage have been reported. The clinical use of Li has caused a decrease in sperm function indices, sexual desires, and the expression of essential genes related to steroidogenesis, followed by disruption of steroidogenesis and spermatogenesis in male rats.[4] Studies have shown that Li has adverse effects on the testicles and semen quality[5,6] through the induction of oxidative stress and dysfunction of mitochondria. In this regard, it is noteworthy that Li may exert severe DNA disorders, break the integrity of the plasma membrane, and denature proteins, thereby causing sperm dysfunction by increasing the level of reactive oxygen species (ROS) in the testis and semen.[4]

Magnesium sulfate ($MgSo_4$) is the second cation in the intracellular compartment and the fourth most abundant cation in the whole body.[7-9] It is known as a strong stimulus for sperm motility and spermatogenesis.[10] Numerous studies show that magnesium has a positive effect on the human reproductive system, such as the quality of semen and fertilization.[6,11,12]

In addition, Mg^{+2} deficiency may affect sperm production, motility, and maturation. There is evidence that infertility is strongly related to the level of magnesium in semen because the energy required for fertilization capacity or sperm motility is produced from Mg^{2+} ATP-dependent ATPase.[12]

Exercise is an important lifestyle factor in promoting general health and fertility throughout life.[13,14] The World Health Organization has recommended that physical activity of longer than 150 min weekly could decrease the risk of reproductive disorders. Numerous studies have shown that in lifestyle-related diseases such as obesity and diabetes, low to moderate-intensity exercise has a positive effect on the quality of semen, leading to an increase in the number of sperm and a reduction of testicular atrophy and inflammation that eventually results in decreasing infertility problems.[15-17]

So far, few studies have been done on infertility caused by Li. On the other hand, Li is a cheap drug available. Considering the attention paid to exercise and receiving supplements to increase the level of public health, this study is planned to determine the protective impacts of $MgSo_4$ administration together with exercise in male Wistar rats.

Materials and Methods

Animal and experimental protocol

Subjects of this study included 72 male Wistar rats, weighing 184.4 ± 7.2 g, being kept at 23–25°C with 12/12-h light/dark cycles, and being acclimatized to the conditions for seven days. The animals were fed with rat chow and water *ad libitum*.

The animals were classified into 12 groups, each including 6 animals; control (saline); magnesium sulfate ($MgSo_4$ 80 mg/kg/day); moderate-intensity training (MIT); magnesium sulfate and moderate-intensity training ($MgSo_4$+MIT); Lithium 10 (Li10 mg/kg/day/IP); Lithium 10 and magnesium sulfate (Li10+$MgSo_4$); Lithium 10 and moderate-intensity training (Li10+MIT); Lithium 10, magnesium sulfate, and moderate-intensity training (Li10+$MgSo_4$+MIT); Lithium 40 (Li40 mg/kg/day/IP); Lithium 40 and magnesium sulfate (Li40+$MgSo_4$); Lithium 40 and moderate-intensity training (Li40+MIT); and Lithium 40, magnesium sulfate and moderate-intensity training (Li40+$MgSo_4$+MIT).

For approximately seven days prior to an 8-week exercise training intervention, the rats in MIT groups were trained, practiced, and got used to the treadmill running – type USD5000, Form USD4500 Shenyang Sino-King Equipment Imp. & Exp. Co., Ltd.[17-19]

Animals in the control group were kept under normal laboratory conditions without exercise for 6 weeks. At the same time, animals in other groups received intraperitoneal injections of Li with doses of 10 or 40 mg/kg/day[20] and $MgSo_4$ with a dose of 80 mg/kg/day[21] for six weeks, between 8:30 and 10:30 in the morning.

Finally, 48 h after the last training session, the blood sample was taken via heart puncture after being anesthetized with ketamine/xylazine at a dose of 75 and 10 mg/kg/IP,[22] respectively, and sacrificed. The blood samples were centrifuged at 6000 rpm for 20 min to separate the serums and kept at −80°C for analysis.[23]

Drugs

Lithium carbonate (CAS-No: 554-13-2) and magnesium sulfate (CAS-No: 10034-99-8) were both purchased from Merck Company in Germany.

Exercise training protocol

Before MIT, the rats experienced a 5-min warm-up and a 5-min cool-down intermittently, at the speed of 10 m/min. In concordance with the previous studies, the exercise training protocols were monitored by the running speed of rats on the treadmill.[18] The MIT consisted of running at a speed of 55% of the maximum capacity over 31 min in the first week and gradually increased until reaching 70% over 46 min at the end of the sixth week.[17,19]

Assessment of FSH, LH, and testosterone

Serum levels of FSH, LH, and testosterone were measured by ELISA kits, Zellbio GmbH, Ulm, Germany.

Sperm characteristics

Once the rats were anesthetized, they were laid on their backside. Next, their skin was disinfected with 70% ethanol and rinsed with distilled water. A sagittal cut of the skin was made about 10 mm long, at the bottom of the scrotum. After testicular exposure, the two caudate epididymitis were observed. Using a 10-mL syringe and a 19-gauge needle puncture, a negative pressure was performed for the aspiration of sperms, in the lower pole of both testicles. Similarly, the needle was pushed in different directions, 4–6 times. The aspirated liquid in the syringe was transferred into a dish filled with 1 ml, 40° saline, to be surveyed for the existence of moving sperm.[24,25]

Sperm count, its concentration, and its progressive and nonprogressive motility were analyzed by means of a computer-aided sperm analysis system (MShot ImageAnalysis System, V1.0).

Histopathologic analysis

After being excised and weighed, the testes were immersed in a formalin solution for histopathological assessments. The tissue samples were embedded in paraffin wax and sectioned at a thickness of 5 μm. The sections were stained with hematoxylin and eosin. Thereafter, in every part of testes tissues, at least 20 seminiferous tubules were microscopically observed at 200 times magnification. After randomly choosing the first seminiferous tubule, the rest were selected clockwise. Histopathological scoring was conducted according to Johnsen scoring method: 10: full spermatogenesis; 9: many late spermatids, but slightly impaired spermatogenesis with disorganized epithelium; 8: a few late spermatids and more than 5 spermatozoa/tubule; 7: many early spermatids, but no late spermatids or spermatozoa; 6: a few early spermatids, but no spermatozoa or late spermatids; 5: many spermatocytes, but no spermatozoa or spermatids; 4: a few spermatocytes, but no spermatozoa or spermatids; 3: only spermatogonia; 2: only Sertoli cells, but no germinal cells; and 1: no seminiferous epithelium.[26]

Statistical analysis

The data are expressed as mean ± SEM. The sperm parameters (count, concentration, progressive, nonprogressive motility), levels of testosterone, LH, FSH, testis and body weights were analyzed by means of one-way analysis of variance followed by the LSD test. Similarly, the groups were compared with the Kruskal–Wallis or Mann–Whitney U tests on the subject of to the testis tissue damage score (TTDS). Using SPSS version 16 (Chicago, IL, USA), $P \leq 0.05$ was assumed statistically meaningful.

Results

The mean Delta Body Weight (g) and Testis Weight/g/100 g Body Weight

In order to compare the weight of the animals, the difference in weight on the first and last day (delta) was compared in different groups [Table 1]. Finally, in the control group, the rats' body weights increased (54.6 ± 7.48 g). The administration of Li with doses of 10 and 40 mg/kg has significantly reduced the weight gain during the study ($P < 0.05$). In the animals receiving Li 10 mg/kg, the administration of $MgSo_4$ (Li10+ $MgSo_4$ group) and MIT (Li10+MIT group) have significantly compensated the lost weight compared to the Li 10 group ($P < 0.05$). However, administrating these two factors together with Li 10 (Li10+$MgSo_4$+MIT group) at the same time did not have any effect on the weight of the animals compared to the Li10 group. Administering $MgSo_4$ together with Li40 (Li40+$MgSo_4$ group) has significantly increased the weight of animals compared to the Li40 group ($P < 0.05$). However, 42 days of exercise alone with Li40 (Li40+MIT group) and the administration of $MgSo_4$ and exercise (Li40+$MgSo_4$+MIT group) simultaneously with Li40 mg/kg did not have any effect on the weight of animals compared to the Li40 group [Table 1]. Likewise, there was not any significant difference in the mean testicular weight/100 g body weight among experimental groups.

Hormonal levels

Serum Testosterone levels

The comparison of testosterone serum levels in the groups shows that the administration of Li only with a dose of 40 mg/kg causes a significant decrease in comparison with that of the control group. The administration of $MgSo_4$ (Li40+$MgSo_4$ group) and MIT (Li40+MIT group) each improved its testosterone serum level in comparison with the Li40 group ($P < 0.05$). However, administrating these two together with Li40 (Li40+$MgSo_4$+MIT group) did not have any effect on serum testosterone levels [Table 2].

Serum LH and FSH levels

A comparison of the serum level of FSH between the groups did not indicate any meaningful difference. At the same time, the results showed that the serum level of LH was significantly reduced only in the Li40 mg/kg group compared to that of the control group. Administering Li40 with $MgSo_4$ (Li40+$MgSo_4$ group) and MIT (Li40+MIT group) each improved LH serum level in comparison with that of the Li40 group. However, the administration of these two together with Li40 (Li40+$MgSo_4$+MIT group) did not have any effect on serum testosterone levels [Table 2].

Sperm parameters

Exposure to doses of 10 and 40 Li caused a significant decrease in sperm parameters. Four categories (quantity, progressive, nonprogressive, and immotile) were considered for sperm motility indexing.

Sperm count (Quantity)

Counting the number of sperms indicates a significant

Table 1: The mean Delta Body Weight (g) and Testis Weight/g/100 g Body Weight

Groups	Delta Body Weight (g)	Testis Weight/g/100 g Body Weight
Control	54.60±7.48	3.80±0.37
$MgSo_4$	18.83±6.68	4.83±0.47
MIT	6.75±3.90	4.40±0.24
$MgSo_4$+MIT	55.50±12.08	4.50±0.50
Li10	23.16±13.11*	5.16±0.40
Li10+$MgSo_4$	58.66±5.30$	4.73±0.42
Li10+MIT	37.66±11.45$	4.00±0.49
Li10+$MgSo_4$+MIT	20.33±6.00	4.33±0.33
L40	9.83±11.40*	4.23±0.27
Li40+$MgSo_4$	18.20±5.88&	3.40±0.74
Li40+MIT	13.16±4.12	3.80±0.21
Li40+$MgSo_4$+MIT	1.83±9.69	4.35±0.42

Means±SEM, the symbol of * indicates significant differences ($P \leq 0.05$) compared to the control group, the symbols of $ and & indicate significant difference from the Li10 mg/kg/d and Li40 mg/kg/d group, respectively. Li=Lithium. $MgSo_4$=magnesium sulfate, Moderate-intensity training=MIT

decrease in their number after the administration of Li10 and 40 mg/kg compared to that of the control group ($P < 0.05$). The performed treatments showed that the administering of $MgSo_4$ (Li10+$MgSo_4$ group) and MIT (Li10+MIT group) each with Li10 had no effect on the number of sperms, but the administration of both simultaneously with Li10 (Li10+$MgSo_4$+MIT group) increased the number of sperms compared to the Li10 group. The interventions performed in this study had no effects on the reduction of sperms induced by Li 40 mg/kg.

Sperm progressive motility

The results indicate a significant decrease in the progressive movement of sperms following the administration of Li40 compared to that of the control group ($P < 0.05$). The administration of Li10 mg/kg decreased the progressive movement, but it did not show a significant difference with that of the control group. Administering $MgSo_4$ with Li40 (Li40+$MgSo_4$ group) did not affect the progressive movement of sperms. However, 6 weeks of MIT with Li40 (Li40+MIT group) has significantly improved their forward movement in the Li40 group. The administration of $MgSo_4$ and MIT (Li40+$MgSo_4$+MIT group) simultaneously with Li40 did not affect the progressive movement [Table 3].

Table 2: Serum concentrations of LH, FSH, and testosterone, respectively

Groups	Testosterone (pg/dl)	LH (pg/dl)	FSH (ng/dl)
Control	218.00±0.54	154.40±0.04	13.00±0.03
$MgSo_4$	202.33±1.29	793.33±4.97	20.33±0.10
MIT	198.75 ± 1.15	70.75±0.21	12.60±0.02
$MgSo_4$+MIT	146.67±0.62	101.66±0.52	13.00±0.47
Li10	209.00±0.55	157.80±0.85	13.20±0.02
Li10+$MgSo_4$	216.33±0.32	123.66±0.92	18.50±0.04
Li10+MIT	198.00±0.46	70.20±0.35	20.50±0.05
Li10+$MgSo_4$+MIT	194.33±0.51	509.33±2.50	15.00±0.03
Li40	45.50±0.10*	14.33±0.08*	15.83±0.32
Li40+$MgSo_4$	82.83±0.37&	129.50±0.41&	16.16±0.33
Li40+MIT	135.25±0.57&	282.80±0.09&	14.51±0.30
Li40+$MgSo_4$+MIT	44.20±0.14	53.40±0.49	15.23±0.46

Means±SEM, the symbol of * Indicates significant differences ($P \leq 0.05$) compared to the control group. The symbol of & indicates significant differences ($P \leq 0.05$) compared to the Li40 mg/kg/d group. Li=Lithium. $MgSo_4$=magnesium sulfate, moderate-intensity training=MIT

Sperm nonprogressive motility

The results indicate a significant increase in the nonprogressive movement of sperms following the administration of Li40 compared to that of the control group. The administration of Li10 had no effect on the movement. Administering $MgSo_4$ with Li40 (Li40+$MgSo_4$ groups) caused a significant decrease in this motility (nonprogressive motility) compared to that of the Li40 group. However, the simultaneous administration of MIT with Li40 (Li40+MIT group), $MgSo_4$ and MIT together with Li40 (Li40+$MgSo_4$+MIT group) had no effects on the nonprogressive movement of sperms [Table 3].

Sperm motility

Investigating sperm motility by immobility index has been compared among different groups. The level of immobility in the control group was about 33.02 ± 2.09 signifying a significant difference with other groups, $P < 0.05$, [Table 3]. Administering Li10 and 40 mg/kg has decreased motility, leading to an increase in the immobility of sperms. In fact, the results show that the exposure to 10 and 40 mg/kg Li caused a significant increase in immovability of sperms (58.66 ± 0.95) and (75.60 ± 4.06), respectively. However, the administration of $MgSo_4$ with Li10 (Li10+$MgSo_4$ group) has no effect on the immovability of sperms while MIT with Li10 (Li10+MIT group) leads to a decrease in their immobility significantly

Table 3: Sperm parameters Quantity, Progressive motility, Nonprogressive motility, Immotile, and Testicular Tissue Damage (TTD)

Groups	Quantity	Progressive %	Nonprogressive %	Immobile %	TTDS
Control	1516.80±85.66	3.20±0.37	27.40±1.50	33.00±2.09	10±0.00
$MgSo_4$	1348.43 ± 42.60	2.43±0.95	32.83±1.27	56.50±1.98	9.2±0.18
MIT	1209.32 ± 21.38	14.40±1.69	30.16±3.31	27.00±2.25	9.50±0.24
$MgSo_4$+MIT	1273.16 ± 83.85	1.83±0.47	23.39±2.70	29.50±1.43	8.50±0.34
Li10	932.66±54.82*	2.66±0.21	28.33±1.54	58.66±0.95*	9.00±0.16
Li10+$MgSo_4$	860.50±15.23	3.33±0.42	33.33±1.40	59.66±2.62	8.20±0.20
Li10+MIT	911.83±20.74	2.83±0.79	35.83±2.95	46.66±2.55$	9.10±0.40
Li10+$MgSo_4$+MIT	1188.80±108.35$	3.00±0.25	25.08±3.42	40.66±1.76$	8.20±0.58
L40	243.45±23.03*	1.20±0.37*	43.00±0.33*	75.60±4.06*	9.80±0.22
Li40+$MgSo_4$	250.80±8.54	2.00±0.00	31.80±1.24&	63.00±1.74&	8.60±0.81
Li40+MIT	160.00±10.96	4.20±1.02&	42.20±3.13	38.40±3.22&	8.62 ± 0.52
Li40+$MgSo_4$+MIT	306.83±12.04	3.66±0.42	39.75±1.50	46.83±1.81&	9.35±0.25

Means±SEM, the symbol of * Indicates significant differences ($P \leq 0.05$) compared to control group, the symbol of $ indicates a significant difference from Li10 mg/kg/d. The symbol of & indicates significant differences ($P \leq 0.05$) compared to the Li40 mg/kg/d group. The symbol of # indicates significant differences ($P \leq 0.05$) compared to Li40+$MgSo_4$ and Li40+MIT groups, Li=Lithium. $MgSo_4$=magnesium sulfate, moderate-intensity training=MIT, and testicular tissue damage score (TTDS)

and improves the movement of sperms [Table 3]. In addition, the coadministration of $MgSo_4$ 80 mg/kg and MIT with Li10 can decrease immobility of sperms in Li10+$MgSo_4$+MIT group; nonetheless, the effect of both together was not greater than that of $MgSo_4$ and MIT individually [Table 3]. Administration of $MgSo_4$ and MIT together with Li40 (Li40+$MgSo_4$ and Li40+MIT groups) has led to a significant decrease in immobility ($P < 0.05$). It should be noted that the effect of the simultaneous administration of MIT and $MgSo_4$ with Li40 (Li40+$MgSo_4$+MIT group) was greater than that of the individual administration and it had a better effect on sperms motility compared to the use of these two separately with Li40 mg/kg (Li40+MgSo4 and Li40+MIT groups) [Table 3].

Histopathologic analysis

A comparison of TTDS in different groups did not show any significant difference among them. The average tissue damage scores in different groups were as follows: Control: 10 ± 00, $MgSo_4$: 9.2 ± 0.18, MIT: 9.50 ± 0.24, $MgSo_4$+MIT: 8.50 ± 0.34, Li10: 9.00 ± 0.16, Li10+$MgSo_4$: 8.20 ± 0.20, Li10+MIT: 9.10 ± 0.40, Li10+$MgSo_4$+MIT: 8.20 ± 0.58, L40: 9.80 ± 0.22, Li40+$MgSo_4$: 8.60 ± 0.81, Li40+MIT: 8.62 ± 0.52, and Li40+$MgSo_4$+MIT:9.35±0.25 [Table 3, Figure 1].

Discussion

The main findings of the present study are as follows:

1) Lithium carbonate with a dose of 40 caused the most damage to the testicular tissue and sperm parameters. 2) It also caused a decrease in the serum level of luteinizing hormones and testosterone. 3) MIT and $MgSo_4$ significantly improved the reproductive performance of the male animals after Li administration.

The findings of the present study are compatible with Toghyani *et al*., who have reported that Li can reduce spermatogenesis and the number of sperms in a dose-dependent manner after 42 days. According to the other studies, Li probably exerts its effects on the reproductive system in different ways, including: 1. Li may act by changing signaling pathways such as the inositol triphosphate cycle (IP3), cyclic AMP (cAMP), and disruption of ATP synthesis.[27] 2. Damages to Leydig cells decrease in testosterone levels. 3. Li decreases sperm motility.[28] 4. Li may also disrupt spermatogenesis by reducing the activity of the hypothalamus-pituitary-gonadal axis. Similarly, several studies indicate that Li can even directly affect the testicular tissue by passing through the blood–testicular barrier. It can disturb the sex cells' growth cycle and their maturation, and eventually reduce the sperm count.[3,29] Decreased sperm motility after Li administration may be caused by inhibition of the activity of cAMP, disruption of the activity of adenylate cyclase enzyme, and reduction of cAMP. cAMP and its related protein kinase stimulate the phosphorylation of dynein and proteins of sperm microtubules and increase their pulsations.[30-32] Furthermore, Li can increase lipid peroxidation and perturb the antioxidant system with the formation of free radicals and ROS.[20]

The present study showed that $MgSo_4$ can compensate for the effects of Li on reproductive performance by improving sperm parameters. Consistent with these results, Asghari *et al.* have reported that $MgSo_4$ improved testicular tissue damage and increased the number of spermatozoa after 42 days. Eghbali M, *et al*. showed that probably $MgSo_4$ exerts its effects by improving the antioxidant status of the testicular tissue, including modulating malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase (GPx).[33] In addition, there is evidence that $MgSo_4$ may change the characteristics of the seminal fluid and increase sperm

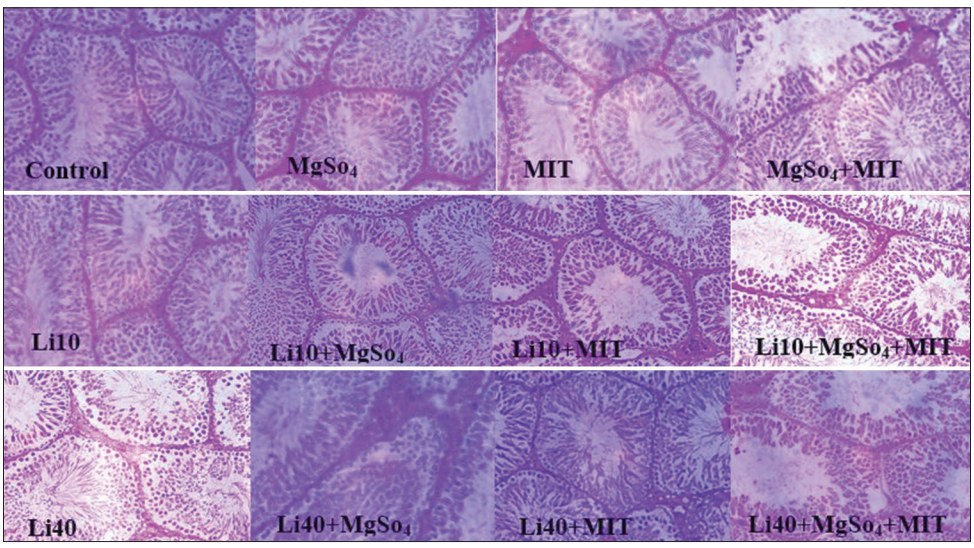

Figure 1: The pathology images (×200) of testis tissue. The groups received saline, $MgSo_4$ 80mg/kg/d, MIT, $MgSo_4$+MIT, Li10 mg/kg/d, Li10+$MgSo_4$, Li10+MIT, Li10+$MgSo_4$+MIT, Li40 mg/kg/d, Li40+$MgSo_4$, Li40+MIT, and Li40+$MgSo_4$+MIT for 42 days

motility. It is reported that the antioxidant capacity of semen is improved with $MgSo_4$ administration; however, reducing the level of magnesium may reduce the antioxidant capacity of semen.[34] Eghbali M and Ścibior A have reported that $MgSo_4$ administration may reduce free radicals in liver cells following the blockage of bile ducts.[34,35] Likewise, the use of $MgSo_4$ in other pathological conditions, such as diabetes, hypertension, and atherosclerosis, induces a strong antioxidant activity, the mechanism of which has not yet been precisely determined.[36]

In the present study, a significant increase in total and free testosterone levels, sperm count and motility, was observed among the rats which underwent moderate-intensity exercise. Numerous studies have similarly shown that moderate-intensity aerobic exercise is connected to improved testis functional parameters such as sperm concentration and hormonal serum levels. In this regard. Gaderpour *et al*. have reported that voluntary exercise effectively and markedly improves the reproductive system and sperm parameters among type-2 diabetic male rats.[37] They suggested that exercise may induce the above-mentioned effects by reducing oxidative stress and modulating the related signaling pathways.[37] Contrary to the results of this study, Hosseini *et al*. have reported that high-intensity intermittent training and low-intensity endurance training did not affect the morphological parameters, concentration, and movement of sperm and DNA integrity.[38] It is possible that exercise could exert its effects through the hypothalamic-pituitary-gonadal axis[38] which results in increased stimulation of Leydig and Sertoli cells, as evidenced by raised levels of LH and FSH hormones in this study.

Li treatment may lead to an increase in the level of ROS and a decrease in antioxidant levels in sperm cells – which may result in oxidative stress. This has traditionally been identified as a significant contributor to the development of sperm abnormalities and damage to sperm DNA in infertile men.[39]

Exercise can stimulate the production of antioxidant and anti-inflammatory molecules that may prevent the occurrence of diseases related to inflammation and oxidative stress. Considering the link between inflammation, oxidative stress, and male infertility, modifying the levels of these markers through exercise or other interventions may have therapeutic benefits.[19,40]

Numerous studies have shown that engaging in moderate-intensity aerobic exercise can have a positive impact on various parameters related to testis function, including sperm concentration and hormonal serum levels. In fact, individuals who have undergone training have been observed to have increased levels of total and free testosterone, as well as improvements in sperm count, motility, and morphology.[41]

Contrary to the expectation of some studies, they have reported harmful or even negative effects of MIT on testicular function. For instance, Safarinejad *et al*. found lower levels of free and total testosterone, LH, and FSH in moderate-intensity trained men, indicating decreased testis function.[42] There are many studies related to the role of exercise in improving infertility. Some studies have reported no significant alterations in FSH and decreased serum levels of LH,[43] while some others have found increased LH serum levels.[44] Probably, possible differences in the duration and frequency of exercise training programs in the studies may explain the contradictory results. Therefore, further research is needed to more certainly approve the effects of exercise on testicular function.

The final result is that the consumption of Li40 mg/kg can cause possible damages to the reproductive system. However, this effect of Li is confirmed by the decrease in the level of LH and testosterone. Although more studies are still needed regarding the effects of MIT and $MgSo_4$ on Li infertility, these two factors probably exert their influence in reducing Li infertility and improving reproductive performance by increasing antioxidant capacity.

Ethics approval and consent to participate

The research project was certified by the Zahedan University of Medical Sciences Ethics Committee (Ethic No. IR.ZAUMS.REC.1401.056).

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Conflicts of interest

There are no conflicts of interest.

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