

Research Article

In Vitro Coexposure of Silymarin with Lithium Chloride Preserves Human Sperm Quality: A Useful Approach to Reduce Oxidative Damages

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Received 13 May 2023; Revised 27 October 2023; Accepted 28 October 2023; Published 27 November 2023

Academic Editor: Muhammad Babar Khawar

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Lithium, a common component in various mood-stabilizing drugs, induces adverse effects on spermatozoa and human fertility. Oxidative stress, as a possible mechanism involved in lithium toxicity, can be reversed using antioxidants. Therefore, in this study, the effects of silymarin as a potent antioxidant were evaluated on human spermatozoa treated with lithium. Human spermatozoa were divided into five groups: (1) spermatozoa at 0 min, (2) spermatozoa at 180 min (control group), (3) spermatozoa treated with lithium chloride (500 μ M) for 180 min, (4) spermatozoa were treated with silymarin (100 μ M) + lithium chloride (500 μ M) for 180 min, and (5) spermatozoa treated with silymarin (100 μ M) for 180 min. Sperm motility and viability, the levels of malondialdehyde (MDA) and ferric reducing antioxidant power (FRAP), and the activity of antioxidant enzymes (catalase, superoxide dismutase, glutathione peroxidase) were evaluated in these groups. The results showed that sperm motility and viability significantly ($p < 0.001$) decreased in the group treated with lithium. In addition, a significant increase ($p < 0.001$) was observed in MDA levels, while FRAP amount and the activity of antioxidant enzymes decreased significantly ($p < 0.001$) in this group. Interestingly, these results were significantly ($p < 0.001$) reversed in the group treated with silymarin + lithium. The finding of the present study showed that oxidative stress plays a crucial role in lithium toxicity in human spermatozoa, and the coapplication of silymarin as a potent antioxidant with lithium can protect human sperm against oxidative stress-induced damage.

1. Introduction

Lithium, the first element in the alkali metals group, is described as a very light metal with high activity [1]. Despite the low amounts of this element in drinking water, some foods, and vegetables, it is widely used in the glass and ceramics industry, and the production of lightweight batteries (used in mobile phones) [2]. In addition, the widespread use of lithium in the pharmaceutical industry is one of the most important lithium applications. In this context, lithium is widely used in mood-stabilizing drugs with anti-suicidal effects [3]. These drugs are also used for the treatment of a variety of neurological damages, such as acute mania and bipolar damage [4]. Long-term use of lithium-containing drugs causes structural and functional disorders in various organs, including the gastrointestinal tract, liver, kidney, nervous system, thyroid gland, and male reproductive system

[5, 6]. The fact that patients with neurological disorders must be exposed to such drugs for a long time and sometimes for the rest of their lives, investigating the harmful effects of lithium on human health and its toxic mechanisms could be important.

Studies have shown that lithium exerts sexual dysfunction in men [7]. In mice, lithium significantly reduced the expression of essential genes associated with steroidogenesis and subsequently impaired spermatogenesis [8]. Infertility has also been reported in male rats due to prolonged (orally) use of lithium, leading to a considerable reduction in spermatogenesis and serum levels of prolactin, testosterone (T), luteinizing hormone, follicle-stimulating hormone, and a significant decrease in the activity of steroid-related genes (-3-HSD and 17 β -HSD). Additionally, birds treated with lithium showed significant degenerative changes in germ cells and the epithelium of seminiferous tubules. Moreover,

lithium impairs sodium/potassium pump function, cell ion balance, and the mitochondria function, which in turn disrupts the spermatogenesis process and reduces sperm viability [8]. Our previous results have also illustrated the destructive effects of lithium chloride on motility and viability in ram sperm [9].

Numerous studies have reported that oxidative stress is the most important mechanism for lithium-induced damage [10–12]. In this context, several studies demonstrated the role of lithium-induced oxidative stress on testicular tissue and the semen of animals and humans [10, 13]. The decreased amount of cytoplasm and the low levels of antioxidant defense enzymes in the sperm, as well as the presence of a large amount of unsaturated fatty acids in the sperm plasma membrane, suggest that spermatozoa have a high susceptibility to damage by oxidative stress [14].

In recent years, the use of antioxidants has been widely studied to compensate for the damaging effects of oxidative stress on male fertility [15, 16]. Therefore, antioxidant therapy might be considered the first clinical treatment for infertile men who suffer from infertility caused by oxidative stress. Silymarin is a polyphenolic flavonoid compound extracted from the seeds of milk thistle (*Silybum marianum*) with several therapeutic properties, including strong antioxidant, anti-inflammatory, anticancer, antianxiety, regulating the activity of the immune system, treatment of infectious and liver diseases, as well as lowering plasma cholesterol [17, 18]. In addition, it acts as a cell membrane stabilizer and cell glutathione (GSH) enhancer and is responsible for the detoxification and elimination of free radicals [19]. Numerous studies have shown the antioxidant effects of silymarin on various tissues and cells, including the liver, spleen, heart, arteries, kidney, and testis [20–25].

Several studies have documented the harmful effects of lithium and the role of oxidative stress in lithium toxicity [10, 12, 26]. In addition, silymarin is known as a potent antioxidant [19, 20, 27]. Therefore, in this study, we aimed to investigate whether oxidative stress is the mechanism involved in lithium toxicity on vital parameters of human spermatozoa and also whether silymarin can attenuate the adverse effects of lithium on these parameters to provide new insight into preventing infertility in the men consuming medicines containing lithium chloride.

2. Materials and Methods

In this experimental study, human sperm samples (30 normospermia donors, 25–40 years old) were obtained from the Academic Center for Education, Culture, and Research, Arak, Iran, and transferred to the biology research laboratory at Arak University. High-quality samples (in terms of number and motility) were then used for all experiments. The experiments were approved by the local ethics committee at Arak University of Medical Sciences, Arak, Iran (IR.ARA-KMU.REC.1397.218).

2.1. Preparation and Grouping of Samples. Sperm samples were mixed 1:1 with a human tubal fluid culture medium containing 0.1% bovine serum albumin and centrifuged at

1,500 rpm for 4 min. The sperm pellet was then mixed with the medium, and the sperm suspension was used for all experiments. Sperm samples were counted according to the World Health Organization guidelines [28] and then separated into sterile Eppendorf tubes (each tube contained 2×10^7 spermatozoa). The tubes were then divided into five groups: (1) spermatozoa at 0 min, (2) spermatozoa at 180 min (control group), (3) spermatozoa treated with lithium chloride (Merck, Germany, $500 \mu\text{M}$) for 180 min, (4) spermatozoa were treated with silymarin (Sigma, USA, $100 \mu\text{M}$) +lithium chloride ($500 \mu\text{M}$) for 180 min. Silymarin was added 15 min before lithium chloride, and (5) spermatozoa treated with silymarin ($100 \mu\text{M}$) for 180 min. Samples in Groups 2–5 were incubated at 37°C for 180 min [29]. To find out the effective concentration for lithium chloride and silymarin, sperm samples were separately treated with lithium chloride (200, 500, and $1,000 \mu\text{M}$) and silymarin (50, 100, and $150 \mu\text{M}$) [30]. After 180 min, sperm viability was assessed by eosin-negrosin staining (see below). Results of sperm viability (data of concentration finding not shown) revealed that the concentrations of 500 and $100 \mu\text{M}$ were effective concentrations of lithium chloride and silymarin, respectively.

2.2. Assessment of Sperm Motility. Sperm motility was evaluated according to the World Health Organization guidelines [28]. Briefly, $10 \mu\text{l}$ of sperm suspension from each group was placed on a slide and evaluated using a light microscope at 400x magnification. At least 200 spermatozoa were counted in five microscopic fields, and the percentage of progressive motility was then reported.

2.3. Assessment of Sperm Viability. Sperm viability was assessed according to the WHO guidelines [28] using eosin-negrosin staining. Briefly, $10 \mu\text{l}$ of sperm suspension and $20 \mu\text{l}$ of eosin (1%) were gently mixed. After 30 s, $30 \mu\text{l}$ of negrosin (10%) was added to the solution, and after mixing, a thin smear of the sample was prepared on the glass slide. Slides were checked using a light microscope at 1,000x magnification. The 200 spermatozoa were counted, and the percentage of live sperm was reported. In this staining, live spermatozoa heads are seen in white and nonliving spermatozoa heads are stained in red color.

2.4. Assessment of Total Antioxidant Capacity. FRAP (ferric reducing antioxidant power) was measured according to Benzie and Devaki's [31] method to evaluate total antioxidant capacity. Briefly, sperm suspensions (2×10^7 spermatozoa) were incubated with trichloroacetic acid (TCA) solution for 15 min and then centrifuged at $1,200 g$ for 10 min. The supernatant was mixed with $1,500 \mu\text{l}$ of FRAP reagent (containing 300 mM acetate buffer, pH 3.6; 10 mM TPTZ solution in 40 mM HCl and 20 mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ solution in the ratio of 10:1:1, respectively) and incubated at 37°C for 4 min. Then, the absorbance of the solution was measured using a spectrophotometer (PG instruments T80 UV/VIS, UK) at 593 nm.

Different concentrations of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (Sigma-Aldrich, USA) were prepared, and then the standard curve was drawn using their obtained absorbance. The regression

equation obtained from the standard curve: $y = 0.11x - 0.0029$, $R^2 = 0.987$ (y : the absorption of the samples and x : the concentration of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$). FRAP levels in the different groups were then calculated and reported as $\mu\text{M Fe}^{+2}$ /spermatozoa.

2.5. Assessment of Lipid Peroxidation. Malondialdehyde (MDA) assay was performed to determine the lipid peroxidation in human spermatozoa. Thiobarbituric acid (TBA) spectrophotometry is used as an indicator for unsaturated fatty acids peroxidation. This test involves the reaction of aldehydes with TBA at 100°C under acidic conditions to produce a pink complex. MDA was measured according to the Partyka et al. [32] method. Briefly, $1,000\ \mu\text{l}$ of TBA solution (containing 15% TBA + 0.375% TCA + 0.25 HCl) was added to the sperm suspension (2×10^7 spermatozoa) and heated at 95°C for 15 min. The tubes were then cooled and centrifuged at 1,000 rpm for 10 min, and finally, the absorbance of the supernatant was read by a spectrophotometer at 535 nm. The MDA concentration of spermatozoa in different groups was calculated using its extinction coefficient ($1.56 \times 10^5\ \text{M}^{-1}\ \text{cm}^{-1}$) and reported as nmol/spermatozoa.

2.6. Assessment of Antioxidant Enzymes Activity. The sperm suspension (2×10^7 spermatozoa) was centrifuged at 3,000 rpm for 10 min, and then $1,000\ \mu\text{l}$ Triton (0.1%) was added to the pellet and mixed (Triton is used as the sperm lysis). The samples were placed at 4°C for 30 min, homogenized on ice, and centrifuged at 1,500 rpm for 4 min. Sperm supernatant (homogenate) was then used to assay the activity of antioxidant defense enzymes.

2.6.1. Catalase (CAT) Activity Assay. The activity of CAT was measured according to Aebi's [33] method, which is designed based on the decomposition of hydrogen peroxide (H_2O_2) by CAT. Briefly, $200\ \mu\text{l}$ H_2O_2 and $400\ \mu\text{l}$ potassium phosphate (K_3PO_4) buffer were added to sperm homogenate ($1,000\ \mu\text{l}$) and mixed. After 1 min, the absorbance was read by a spectrophotometer at 240 nm, and the CAT activity was expressed as U/spermatozoa.

2.6.2. Superoxide Dismutase (SOD) Activity Assay. The activity of SOD was measured using tris buffer and pyrogallol according to the Marklund [34] protocol. In this method, rapid autooxidation of pyrogallol in an aqueous or alkaline solution is used to measure SOD activity. This enzyme prevents pyrogallol autooxidation, and this principle is used to determine the concentration of the enzyme. In summary, two groups of samples were used in this experiment: the control sample ($2,900\ \mu\text{l}$ tris buffer + $100\ \mu\text{l}$ pyrogallol) and the test sample ($100\ \mu\text{l}$ sperm homogenate + $2,800\ \mu\text{l}$ tris buffer + $100\ \mu\text{l}$ pyrogallol). The absorption of each group was read by a spectrophotometer (PG instruments T80 UV/VIS, UK) at 240 nm. The activity of SOD was calculated using the A-B/A \times 50 (100×10) formula and expressed as U ml^{-1} . (Absorbance reading of control = A and absorbance reading of test sample = B)

2.6.3. Glutathione Peroxidase (GPx) Activity Assay. The activity of GPx was measured using the Rani et al. [35]

TABLE 1: Evaluation of motility and viability in human spermatozoa in the different groups.

Groups	Sperm progressive motility (%)	Sperm viability (%)
0 min	73.5 ± 1.23^a	85.1 ± 0.75^a
Control	72.0 ± 0.93^a	78.5 ± 1.20^a
Lithium chloride	43.3 ± 2.13^b	41.5 ± 0.98^b
Silymarin + lithium chloride	76.5 ± 1.06^a	73.5 ± 1.03^a
Silymarin	74.8 ± 0.82^a	79.3 ± 0.26^a

Data are presented as means \pm standard deviation. The means with the different letters are significantly different (one-way ANOVA, Tukey's test, $n = 6$ for each group, $p < 0.05$).

method. In this method, GPx oxidizes GSH and simultaneously reduces hydrogen peroxide to water. Briefly, $200\ \mu\text{l}$ of Tris-HCl (40 mM) was mixed with $200\ \mu\text{l}$ of ethylenediaminetetraacetic acid (40 mM) and $100\ \mu\text{l}$ of sodium azide (NaN_3 ; 10 mM) and then $200\ \mu\text{l}$ of the sperm homogenate, $200\ \mu\text{l}$ of reduced GSH (4 mM) and $100\ \mu\text{l}$ of H_2O_2 (2.5 mM) was added and incubated at 37°C for 10 min. Then, $500\ \mu\text{l}$ TCA (10%) was added and centrifuged at 2,000 rpm for 10 min. The absorbance of the supernatant was then read using a spectrophotometer at 340 nm, and the activity of the GPx enzyme was expressed as U/spermatozoa.

2.7. Statistical Analysis. Statistical analysis was performed using the SPSS software (version 16.0, SPSS Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was used for comparison between groups. The normal distribution of data was checked using the Kolmogorov-Smirnov test (K-S test). A $p < 0.05$ was considered statistically significant. The data were represented as means \pm standard deviation.

3. Results

3.1. Evaluation of Sperm Motility (Progressive Motile Sperm; PMS). In the group treated with lithium chloride ($500\ \mu\text{M}$), the percentage of PMS significantly ($p < 0.001$) decreased compared to the control group. While silymarin was able to significantly ($p < 0.001$) compensate for the percentage of PMS in the group treated with silymarin ($100\ \mu\text{M}$) + lithium chloride ($500\ \mu\text{M}$) compared to the lithium chloride group. Application of silymarin alone had no significant ($p > 0.05$) effect on sperm motility compared to the control group (Table 1).

3.2. Evaluation of Sperm Viability. In the group treated with lithium chloride ($500\ \mu\text{M}$), sperm viability significantly ($p < 0.001$) decreased compared to the control group. Whereas, in the group treated with silymarin ($100\ \mu\text{M}$) + lithium chloride ($500\ \mu\text{M}$), silymarin could significantly ($p < 0.001$) reverse the effect of lithium chloride on sperm viability. Application of silymarin alone had no significant ($p > 0.05$) effect on sperm viability compared to the control group (Table 1 and Figure 1).

3.3. Evaluation of Total Antioxidant Capacity. The levels of FRAP in the lithium chloride group ($500\ \mu\text{M}$ for 180 min)

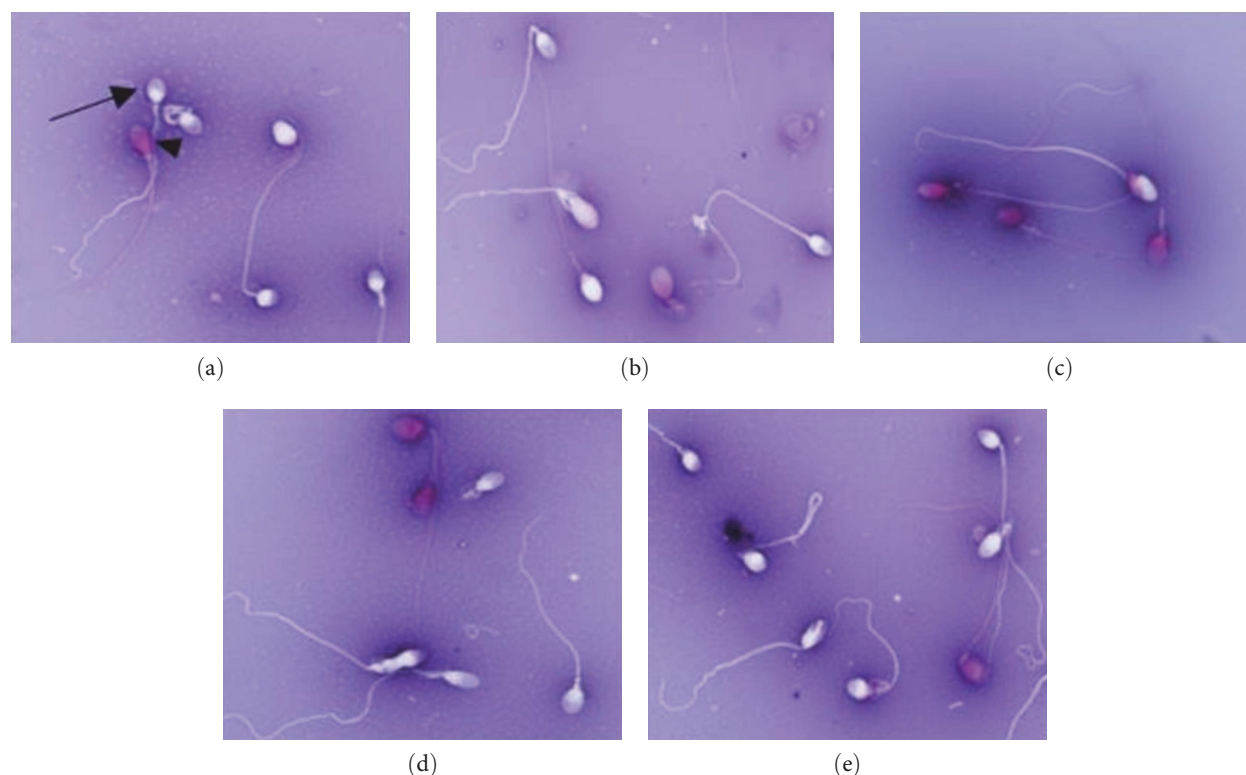


FIGURE 1: Evaluation of human sperm viability in different groups using eosin-negrosin staining method: (a) spermatozoa at 0 min; (b) control group; (c) spermatozoa treated with lithium chloride ($500 \mu\text{M}$); (d) spermatozoa treated with silymarin ($100 \mu\text{M}$) + lithium chloride ($500 \mu\text{M}$); (e) spermatozoa treated with silymarin ($100 \mu\text{M}$). Arrow: live spermatozoa with a white head. Arrowhead: dead spermatozoa with red head. Magnification: 1,000x.

TABLE 2: The levels of total antioxidant capacity as measured by FRAP (ferric reducing antioxidant power) and lipid peroxidation as measured by MDA (malondialdehyde) in human spermatozoa in the different groups.

Groups	FRAP ($\mu\text{mol/sperm}$)	MDA (nmol/sperm)
0 min	0.132 ± 0.001^a	0.905 ± 0.001^a
Control	0.126 ± 0.006^a	0.765 ± 0.011^a
Lithium chloride	0.012 ± 0.010^b	2.156 ± 0.021^b
Silymarin + lithium chloride	0.122 ± 0.002^a	0.515 ± 0.001^a
Silymarin	0.128 ± 0.006^a	0.733 ± 0.005^a

Data are presented as means \pm standard deviation. The means with the different letters are significantly different (one-way ANOVA, Tukey's test, $n = 6$ for each group, $p < 0.05$).

were significantly reduced ($p < 0.001$) compared to the control group. In the group treated with silymarin ($100 \mu\text{M}$) + lithium chloride ($500 \mu\text{M}$), silymarin could significantly ($p < 0.001$) elevate FRAP amount compared to the lithium chloride group. The application of silymarin alone had no significant effect on FRAP levels compared to the control group (Table 2).

3.4. Evaluation of Lipid Peroxidation. The levels of MDA in the lithium chloride group ($500 \mu\text{M}$ for 180 min) significantly ($p < 0.001$) increased compared to the control group. In the group treated with silymarin ($100 \mu\text{M}$) + lithium

chloride ($500 \mu\text{M}$), silymarin was able to significantly ($p < 0.001$) decrease the levels of MDA in human sperm compared to the lithium chloride group. The application of silymarin alone had no significant effect ($p > 0.05$) on MDA levels compared to the control group (Table 2).

3.5. Evaluation of the Activity of Antioxidant Defense Enzymes.

The activity of CAT, SOD, and GPx in human sperm significantly ($p < 0.001$) dropped in the group treated with lithium chloride ($500 \mu\text{M}$ for 180 min) as compared with the control group. In the groups treated with silymarin ($100 \mu\text{M}$) + lithium chloride ($500 \mu\text{M}$), silymarin could significantly ($p < 0.001$) increase the activity of these enzymes compared to the lithium chloride group. Application of silymarin alone had no significant effect ($p > 0.05$) on the activity of these enzymes compared to the control group (Table 3).

4. Discussion

The present study demonstrated that lithium, by affecting the antioxidant defense system and the induction of lipid peroxidation, reduced motility and viability (as important parameters in male fertility) in human spermatozoa. While silymarin, as a potent herbal antioxidant, was able to neutralize the destructive effects of lithium on these cells. Lithium has been used in bipolar depression medicines for over 50 years [36]. Despite the wide use of such drugs as mood stabilizers, their side effects on different organs are

TABLE 3: Evaluation of catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) activity in human spermatozoa in the different groups.

Groups	CAT (U/sperm)	SOD (U/sperm)	GPx (U/sperm)
0 min	7.006 ± 0.01 ^a	4.331 ± 0.04 ^a	30.105 ± 1.01 ^a
Control	6.818 ± 0.02 ^a	4.145 ± 0.28 ^a	26.918 ± 0.89 ^a
Lithium chloride	5.241 ± 0.01 ^b	3.080 ± 0.01 ^b	7.715 ± 2.13 ^b
Silymarin + lithium chloride	6.560 ± 0.04 ^a	3.980 ± 0.04 ^a	22.771 ± 1.32 ^a
Silymarin	6.975 ± 0.03 ^a	4.135 ± 0.05 ^a	28.470 ± 0.58 ^a

Data are presented as means ± standard deviation. The means with the different letters are significantly different (one-way ANOVA, Tukey's test, $n = 6$ for each group, $p < 0.05$).

controversial [37]. Several lines of studies illustrated that lithium is widely distributed in tissues after oral usage. Moreover, its concentration in semen has been reported to double the blood [38]. In this context, decreased sperm motility has been reported in patients who consume drugs containing lithium [39]. However, mechanisms involved in lithium toxicity in the human reproductive system are still under discussion. In this way, a positive correlation between oxidative stress and lithium toxicity has been reported [10].

In recent years, oxidative stress has been proposed as one of the most important mechanisms involved in male infertility [40]. Studies have shown that many drugs can affect the production and quality of sperm by inducing oxidative stress, leading to male infertility [41]. In accordance with this, oxidative stress was reported as the main cause of testis damage and infertility in rats treated with lithium (a common drug for bipolar disorder treatment) [12]. Human spermatozoa are very sensitive to reactive oxygen species (ROS) due to a moderate antioxidant defense system and also high amounts of oxidizable substrates [42]. The presence of polyunsaturated fatty acids (PUFAs) such as linolenic, arachidonic, and decahexanoic acids (DHA) in the plasma membrane of human spermatozoa increases the susceptibility of these cells to free radicals. DHA is the predominant fatty acid in the sperm plasma membrane and constitutes more than 50% of all membrane PUFAs [43]. This fatty acid not only plays an important role in regulating the membrane fluidity but also, due to the presence of numerous carbon-carbon double bonds, it acts as the main substrate for ROS attack and the initiation of peroxidation [44]. The cascade of lipid peroxidation reactions forms various products, including some sort of highly reactive lipid aldehydes such as MDA. Under normal conditions, these products are neutralized by the antioxidant defense enzymes [45]. However, the production of large amounts of oxidative markers, more than the capacity of the antioxidant defense system, leads to the accumulation of these products and sperm damage. The excessive accumulation of these products negatively affects sperm motility and viability [46]. In addition, the peroxidation of membrane lipids catabolizes and releases the membrane phospholipids. This causes an abnormal increase in the membrane fluidity, resulting in permeability to ions, as well as dysfunction of

membrane proteins and receptors [47]. Loss of sperm plasma membrane integrity is closely related to decreased sperm motility [48]. This sperm parameter is also affected by changing pH and ion concentration. Abnormal increased permeability to ions impairs the integrity of the sperm plasma membrane and disrupts the function of pumps and ionic channels, decreasing sperm motility [49]. Furthermore, oxidative stress can induce lipid peroxidation in organelles, including mitochondria, to reduce mitochondrial membrane potential and the production of adenosine triphosphate (ATP) required for sperm motility and viability [50]. Disturbance in the structure and function of mitochondria also increases the production of free radicals and induces oxidative stress, leading to more sperm damage [51]. Lithium can induce toxicity in mitochondria through different pathways. This toxicant can easily enter the mitochondria matrix and interact with the mitochondria electron transport chain. This, in turn, disrupts the mitochondria membrane potential and ATP production [52]. Lithium is also able to increase mitochondria membrane permeability and facilitate the release of various mediators of cell death into the cytoplasm [53]. These events can eventually lead to cell death, reducing sperm motility and viability.

The male reproductive system produces antioxidant enzymes that can defend sperm against ROS attack. GPx, CAT, and SOD are known as the most abundant antioxidant enzymes in semen. The deficiency of these enzymes, due to the high amount of ROS, leads to the aggravation of oxidative factors and an increase in lipid peroxidation [54]. In the present study, it is reasonable to assume that lithium chloride could exert its toxic effects on these vital sperm parameters by inducing oxidative stress and/or suppression of the antioxidant defense system. To test this hypothesis, we evaluated several specific indices of oxidative stress in the human spermatozoa treated with lithium chloride. This toxicant increased the level of MDA (as a marker for lipid peroxidation) and decreased the total antioxidant capacity (as measured by FRAP) as well as the activity of antioxidant defense enzymes (GPx, CAT, and SOD). Consistent with our results, lithium significantly reduced the activity of enzymatic antioxidants in the testes and heart, as well as FaDu (ATCC HTB-43) and Vero (ECACC No. 84113001) cell lines [12, 55]. Considering the low amounts of the sperm cytoplasm and the insufficient levels of antioxidants in the spermatozoa, these cells are very sensitive to the oxidative stress. Therefore, even low amounts of toxic agents could induce destructive effects on the sperm and decrease its quality. Our finding also showed that in the silymarin + lithium chloride group, silymarin not only improved the adverse effects of lithium on the vital sperm parameters but also reversed the oxidative stress indexes measured in this study. These findings could support the possibility that the harmful effects of lithium on sperm viability and motility are induced through the induction of oxidative stress. Silymarin is a polyphenolic flavonoid with antioxidant properties, and the presence of a methoxy group (OCH₃) attached to one of the phenolic rings enhances its antioxidant properties. It acts as a cell membrane stabilizer and cell GSH enhancer and is responsible for detoxifying and eliminating free radicals [56]. Silymarin in the form of livergol capsules is used for the treatment of some diseases, including liver diseases,

and no harmful side effects have been reported so far [27]. Therefore, we suggest that this antioxidant can be prescribed as a therapeutic supplement to reduce the side effects of lithium and increase the chance of fertility in patients who consumed drugs containing lithium. Although these results need to be confirmed *in vivo* and patients consumed lithium. We hope that these results can eventually provide a clinical application against these drugs.

5. Conclusion

The present study showed that lithium chloride impairs sperm motility and viability by inducing oxidative stress, and silymarin, as a potent antioxidant, improves the antioxidant status and reverses the adverse effects of oxidative stress on human spermatozoa.

Data Availability

Data are available on request.

Conflicts of Interest

The authors confirm that there are no conflicts of interest in this study.

Acknowledgments

This study was supported by a grant from the Arak University.

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