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# Moderate-intensity training can ameliorate the process of cardiac apoptosis induced by lithium drug consumption in male Wistar rats

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ABSTRACT

*Background and objective:* Lithium medication, given its significant role in the treatment or reduction of psychiatric disorders, may exert adverse effects on cardiac tissue. Therefore, this study aimed to investigate the effects of different exercise training intensities on the process of cardiac apoptosis and serum levels of cardiac troponin I (cTnI) resulting from lithium administration in male Wistar rats.
*Methodology:* In the present experimental study, 35 male Wistar rats were randomly divided into five groups (n=7); Control (CTL), Lithium (Li), High-Intensity training + lithium (HIT-Li), Moderate-Intensity training + lithium (MIT-Li), and Low-Intensity training + lithium (LIT-Li). Lithium drug (dose of 40 mmol/kg dry food weight) and exercise training (5 days per week) were administered for eight weeks. Serum levels of cTnI, mRNA expression of Bcl-2, Bax, and Caspase-3, and histopatholigical changes were assessed by using the ELISA method, Real-Time PCR, and H&E staining, respectively.
*Results:* The expression of the Bcl-2 gene was significantly increased in the LIT-Li group compared to the Li group ($P = 0.003$). Serum levels of cTnI were considereably higher in the Li group compared to the MIT-Li group ($P = 0.0001$). The expression of the Bax gene, in the LIT-Li, HIT-L, and Li groups, significantly increased compared to the MIT-Li group ($P = 0.0001$). Histopathological scores decreased in MIT-Li compared to Li group ($P = 0.001$).
*Conclusion:* It seems that among different exercise intensities, the greatest protective effect against lithium consumption can be observed with moderate exercise intensity, which may potentially modulate factors influencing cardiac apoptosis and reduce lithium toxicity in the cardiac tissue of rats.

## 1. Introduction

Cardiovascular disease is the most common cause of mortality among individuals with psychiatric disorders, and this shows that people with mental illness are more likely to have higher blood pressure fluctuations, which can lead to an increase in cardiovascular disease [40]. Lithium is the first-line treatment option for individuals with acute bipolar disorder in adults [33]. However, this drug has a limited therapeutic index; meaning, the range between toxic and therapeutic doses is narrow, leads to numerous side effects and often organ toxicity, and causes concurrent diseases such as congestive heart failure and liver cirrhosis [1].

The cardiac toxic effects of lithium range from simple electrocardiogram (ECG) abnormalities to dysrhythmias, cardiomyopathy, and even acute myocardial infarction (AMI) [1]. Due to the similarity with sodium, lithium probably replaces sodium and disrupts action potential generation in nerve cells [3]. Lithium is one of the drugs that can modulate apoptosis-regulating proteins both in the mitochondria and in the endoplasmic reticulum network [1]. Apoptosis is a physiological program of cell collapse, which controlled specifically by a group of B-Cell Lymphoma-2 (Bcl-2) family, consisting of the anti-apoptotic protein opposing the pro-apoptotic Bcl-2 family is placed [24]. Bax

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(Bcl 2-like Protein 4) is also another key protein in the regulation of apoptosis. Bcl-2 is an anti-apoptotic protein, the increased expression of which protects cardiac cells from cell death, whereas Bax is a pro-apoptotic protein and is expressed in large amounts during apoptosis [24]. Finally, apoptotic signals activate caspases such as caspase-3 leading to probable cell death [21].

Cardiac troponin I is a regulatory protein of the myocardial contractile system [44]. Cardiac troponin I is considered the gold standard for diagnosing myocardial injury in patients with chest pain [14,44]. Troponins have prognostic significance in partial myocardial damage seen in patients with unstable coronary artery disease and provide valuable clinical management insights [14,44,47]. Other studies have also demonstrated adverse physiological responses, including increased secretion of cardiac troponin I (cTnI), from cardiac tissue in response to high-intensity training [7]. Although the beneficial effects of exercise are clear, the effects of exercise intensity and resultant exercise preconditioning (EP) effects on cardiac ischemia and cardiac physiological responses and functions are uncertain. A specific recommendation for HIIT-Rx or LIIT-Rx to reduce or prevent undesirable health risks is challenging, due to the various combinations that may manipulate components of HIIT and LIIT. These factors include: the number of interval sessions, intensity and duration of each bout, types of recovery periods, and training protocol duration [7].

In recent decades, exercise training become a keen interest among sports researchers [10]. Physical activity is a relatively low-risk and accessible method to reduce physical health and weight health problems in individuals with severe mental illness. Obstacles to physical activity implementation in treating severe mental illnesses include lack of time, difficulty in starting physical activity due to lack of energy, low motivation, and fatigue. exercise interventions can improve cardiovascular fitness in an individual with depression [16,19]. Studies have shown that moderate and continuous exercise likely reduces apoptosis in various tissues [15]. In this regard, McMillan and colleagues reported that six weeks of endurance training reduces DNA fragmentation, cytochrome C release, and Bax protein [30]. Kwak also stated that endurance training reverses apoptotic signaling, processes and suggests that endurance training protects against apoptosis in the heart [22]. Research has shown that people with severe mental illness are at higher risk of physical health complications, particularly cardiovascular disease, and are more likely to be physically inactive. Medications also contribute to increased cardiovascular and metabolic risk, which is usually exacerbated by the use of antipsychotics [16].

Studies show that lithium is a toxic agent for cardiac muscle hence the likelihood of cardiac apoptosis due to high doses of this drug. considering the importance of exercise training and the role of cardiac muscle in health, this issue poses a serious challenge that can captivate the attention of researchers. Therefore, our aim in this study is to investigate whether exercise training in different intensities can eliminate or limit the toxic effects of lithium on cardiac apoptosis.

## 2. Materials and methods

This study was an experimental type and involved a total of 35 male Wistar rats aged eight weeks with an average weight of 200±10 g, purchased from the Physiology Research Center of Kerman. The animals were kept in the animal house conditions at a temperature of 22±2°C under controlled conditions of 12:12-hours of light-dark cycle and 50 % ±10 humidity, in special cages. The animals were acclimatized into the laboratory environment for one week and then randomly divided into five equal groups (n=7); Control, Control with Lithium, High-intensity training with Lithium, Moderate-intensity training with Lithium, and Low-intensity training with Lithium. All animal experiments were conducted in accordance with animal protection policies and approved by the Research Ethics Committee of Shahid Bahonar University of Veterinary Medicine, Kerman (IR.UK.VETMED.REC.1401.007).

### 2.1. Loading lithium

The Control group animals were fed with special rodent chow (Pellet, Javaneh Khorasan Company) and water. The groups receiving lithium were given lithium in combination with chow (dose of 40 mmol/kg dry weight of chow) [23]. To prepare lithium-containing chow, one kilogram of special dry rat food was mixed with 450cc of water and 40 mmol of lithium solution and kneaded. Subsequently, using a special rodent food preparation device, it was pelletized back into Pellet form and dried in a sterile environment [34].

### 2.2. Training protocol

Before implementing the exercise protocol, all animals underwent a two-weeks familiarization period (five days a weekon the of treadmill (Tajhiz-Gostar Omid Iranian). The identification program included running at a speed of 13 m per minute with a 0 % slope for 15 minutes. Maximum speed ($V_{max}$) was measured to estimate $V_{O2max}$. Therefore, all the rats underwent an incremental exercise test, where they were placed on the treadmill and warned up for two minutes at eight meters per minute every two minutes until exhaustion (defined as the inability to maintain speed for three to five minutes) [35]. Three minutes after the incremental exercise, blood sampling was done from each rat to confirm the incremental test. So, blood lactate levels were measured using the Cobas Integra kit and Integra device, with levels above 6 mmol/l considered as reaching the target maximum speed [27].

The main exercise protocol with increased speed and duration lasted for eight weeks (five days a week) and due to the application of three different exercise intensities, the exercise protocol for each group was iso distance-based, meaning that at the end of the eighth week, all the rats would covere the same distance.

#### 2.2.1. High-Intensity training with Lithium (HIT-Li)

In this group, the duration of training in the first and second week was 18×15 m/min and at the end of the eighth week, it was 13×33 m/min (Table 1).

#### 2.2.2. Moderate-Intensity training with Lithium (MIT-Li)

In the first and second week of this training intensity, the rats were placed on a treadmill with a speed and duration of 14×19 m/min. At the end of the eighth week, this training intensity reached 18×24 m/min (Table 2).

#### 2.2.3. Low-Intensity training with Lithium (LIT-Li)

In the first two weeks, this group started with an intensity of 9 m/min for 30 minutes, and at the end of the eighth week, the duration and intensity of the exercise ended at 27×16 m/min (Table 3).

### 2.3. Histopathological study

The hearts were removed, washed with saline, fixed with 10 % buffered formalin, and embedded in paraffin. Slides were prepared and stained with hematoxylin and eosin (H&E) and examined microscopically by two pathologists blinded to the animal groupings. The lesions were graded as 0: none, no damage or inflammatory processes; 1: minimum, focal myocyte damage; 2: mild, small multifocal degeneration with a slight degree of inflammatory

processes; 3: moderate, extensive myofibrillar degeneration and/or diffuse inflammatory processes; and 4: severe, necrosis with diffuse inflammatory processes [17].

**Table 1**
HIT protocol.

| **Weeks** | 1,2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|
| **Distance (m)** | 270 | 308 | 325 | 364 | 403 | 416 | 429 |

**Table 2**
MIT protocol.

| Weeks | 1,2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|
| Distance (m) | 266 | 306 | 323 | 357 | 391 | 414 | 432 |

**Table 3**
LIT protocol.

| Weeks | 1,2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|
| Distance (m) | 270 | 308 | 324 | 364 | 400 | 416 | 432 |

### 2.4. Sampling method

48 hours after the last training session, the rats were anesthetized by intraperitoneal injection of ketamine (90 mg/kg) and xylazine (10 mg/kg). Immediately after anesthesia, the cardiac muscle was dissected, washed with saline solution, placed in tubes, and transferred to −80°C for subsequent analysis [29]. In addition, blood sample was taken from all animals, centrifuged and serum were stored at −20°C for a maximum of 2 weeks until troponin I, a biochemical marker of myocardial injury, was measured by relative Kit (Roche company, cobas c303, 0766062) [32].

### 2.5. Real-time PCR method

For mRNA extraction, the Sinapur-RNA-Cell Culture Tissue extraction kit (Sinaclone) was used. The samples were taken out of the freezer and placed on ice. Approximately 35 mg of cardiac tissue was powdered using a mortar and pestle in liquid nitrogen and homogenized in 400 µl Lysis Solution Buffer. After one minute, 300 µl Precipitation Solution was added to the solution and after shaking, the desired solution was transferred to the specific columns. The columns were centrifuged in a refrigerated centrifuge (model 5430 R, Eppendorf, Germany), at 12,000 g for one minute. The aqueous phase of the samples was extracted and 400 µl Wash I was added to each column, which was centrifuged again at 12000 g for one minute and the aqueous phase was extract again. In the next step, 400 µl Wash II was added to each column and this process was repeated twice. The columns were then centrifuged at 12,000 g for two minutes to dry. After this step, the columns were transferred to RNase-free microtubes (1.5 ml) and 50 µl of distilled water at 55°C was added to each column. The microtubes were incubated in a Dry Bath at 55°C for 15 minutes and immediately centrifuged at 12,000 g for one minute. Finally, the columns were removed from the microtubes and RNA was extracted according to the manufacturer's instructions and cDNA synthesis was performed [29]. cDNA synthesis was carried out using the cDNA Synthesis Kit from Parstous following the manufacturer's instructions. Genes expression was measured by Real-Time PCR method with the applied Biosystems Step One Plus Real-Time PCR system, and quantification was done using the $2^{-\Delta\Delta Ct}$ formula. The PCR program included an initial denaturation step at 95°C for 3 minutes, followed by denaturation at 95°C for 22 seconds and the annealing temperature of the primers, each cycle is considered for 30 seconds (40 cycles). The 18S gene was used as a reference gene for relative expression measurement and for specific amplification control from the melting curve analysis. The sequence of primers used in the research is reported in (Table 4) [6].

**Table 4**
Primer Sequence.

| Genes | Forward Primer | Reverse Primer |
|---|---|---|
| Bcl−2 | ATGTGTGTGGAGAGCGTCAACC | GCATCCCAGCCTCCGTTATC |
| Bax | CCTTTTCTACTTTGCCAGCAAAC | GAGGCCGTCCCAACCAC |
| Caspase−3 | TACCCTGAAATGGGCTTGTGT | GTTAACACGAGTGAGGATGTG |
| 18S | GCAATTATTCCCCATGAACG | GGCCTCACTAAACCATCCAA |

### 2.6. Data analysis method

All data are expressed as mean ± standard deviation (Mean ± SD). The Shapiro-Wilk test was used to determine data normality and the One-Way ANOVA and Tukey's post-hoc test were used to determine differences between groups. Histopathological changes are reported qualitatively as the number of animals with different grades of myocardial lesions in each group and statistical analysis was performed using the non-parametric Kruskal-Wallis test, while pairwise differences were determined with Mann-Whitney U tests. A significance level of $P < 0.05$ was considered. All statistical analyses were performed using SPSS software version 23.

## 3. Results

The Li group showed a significant decrease in Bcl-2 gene expression compared to the CTL group ($P = 0.003$). Additionally, there was a significant increase in the gene expression of Bcl-2 in the LIT-Li and MIT-Li compared to the Li group ($P = 0.002$, Fig. 1). A significant increase in Bax gene expression was observed in the Li group compared to the CTL group ($P = 0.0001$) (Fig. 2). Furthermore, the LIT-Li ($P = 0.0001$), Li ($P = 0.0001$) and HIT-Li ($P = 0.0001$) groups also demonstrated a significant increase in Bax gene expression compared to the MIT-Li group ($P < 0.05$).

The LIT-Li, MIT-Li and HIT-Li groups exhibited a significant decrease in gene expression of Caspase-3 compared to the Li group ($P = 0.0001$) (Fig. 3).

There was a significant increase in cardiac troponin I (cTnI) levels in the Li group compared to the CTL group ($P = 0.001$). The HIT-Li group shows a significant increase in cTnI levels compared to the CTL group ($P = 0.021$). Additionally, a significant increase in cTnI levels was observed in the LIT-Li group compared to the CTL group ($P = 0.022$). On the other hand, the MIT-Li group exhibited a significant decrease in cTnI levels compared to the Li, HIT-Li, and LIT-Li groups ($P < 0.05$) (Fig. 4).

Table 5 shows the effects of different intensities of training on myocardial tissue injuries. In the MIT-Li group, the rate of tissue damage was less in comparison to the LIT-Li, HIT-Li and Li groups; thus, moderate-intensity training induced a higher tolerance against lithium toxicity (Table 5).

## 4. Discussion

The current study demonstrated that moderate-intensity training remarkably altered the expression of pro-apoptotic genes and positively

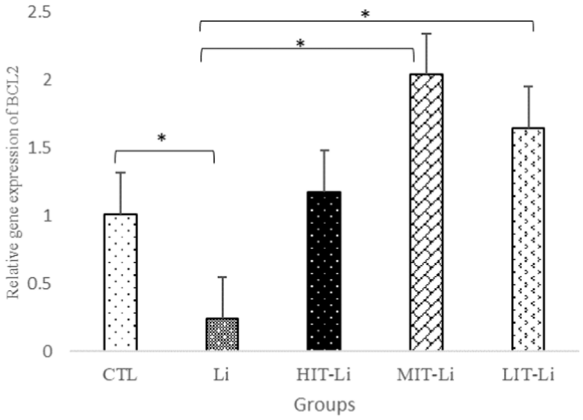


**Fig. 1.** Relative gene expression of Bcl-2 in 5 studied groups including control (CTL), Control + Lithium (Li), High-Intensity training + Lithium (HIT-Li), Moderate-Intensity training + Lithium (MIT-Li), Low-Intensity training + Lithium (LIT-Li). Data are expressed as Mean±SD, $p < 0.05$ was considered as significant difference. * Statistically significant compared to Control+Lithium.

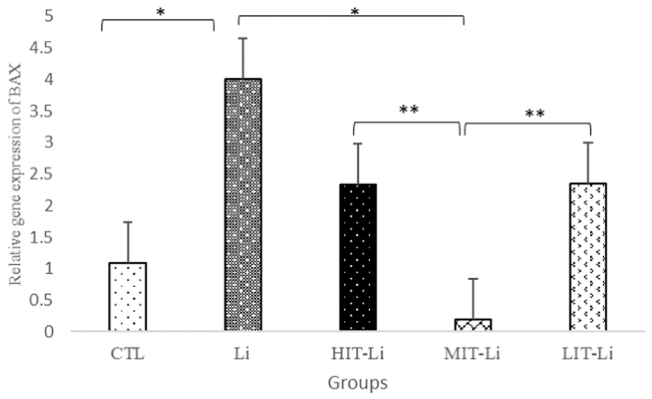


**Fig. 2.** Relative gene expression of Bax in 5 studied groups including control (CTL), Control+Lithium (Li), High-Intensity training+Lithium (HIT-Li), Moderate-Intensity training+Lithium (MIT-Li), Low-Intensity training+Lithium (LIT-Li). Data are expressed as Mean±SD, $P < 0.05$ was considered as significant difference. * Statistically significant compared to Control-Lithium, ** statistically significant compared to MIT-Li.

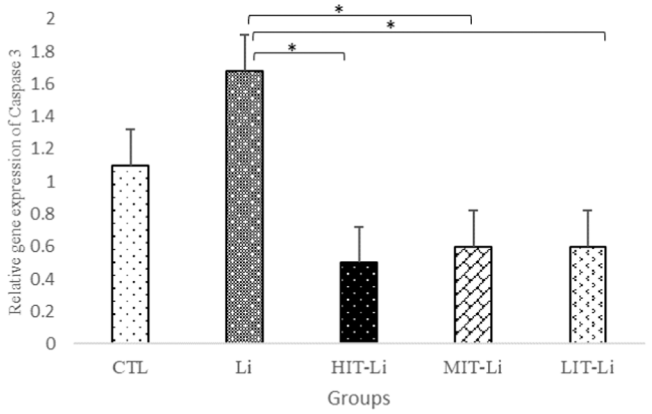


**Fig. 3.** Relative gene expression of Caspase-3 in 5 studied groups including control (CTL), Control+Lithium (Li), High-Intensity training+Lithium (HIT-Li), Moderate-Intensity training+Lithium (MIT-Li), Low-Intensity training+Lithium (LIT-Li). Data are expressed as Mean ± SD, $P < 0.05$ was considered as significant difference. * Statistically significant compared to Control-Lithium.

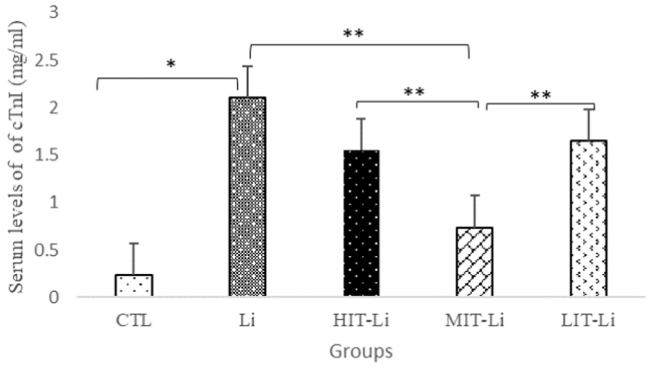


**Fig. 4.** Serum levels of cTnI in 5 studied groups including control (CTL), Control+Lithium (Li), High-Intensity training+Lithium (HIT-Li), Moderate-Intensity training+Lithium (MIT-Li), Low-Intensity training+Lithium (LIT-Li). Data are expressed as Mean ± SD, $P < 0.05$ was considered as significant difference. * Statistically significant compared to Control-Lithium, ** statistically significant compared to MIT-Li.

**Table 5**
Number of Animals Showing Different Pathology Scores in Various Groups.

| Group | Myocardial lesion score | | | | | | |
|---|---|---|---|---|---|---|---|
| | N | 0 | 1 | 2 | 3 | 4 | Mean rank |
| CTL | 8 | 7 | - | - | - | - | 4.50 |
| Li | 8 | - | - | - | 2 | 5 | 26.38[a] |
| HIT-Li | 8 | - | - | 2 | 3 | 2 | 21.88[a] |
| MIT-Li | 8 | - | 4 | 2 | 1 | - | 13.25[a,b,c] |
| LIT-Li | 8 | - | - | 2 | 2 | 3 | 22.56[a] |

CTL: Control, LIT-Li: Low-Intensity training+Li, MIT-Li: Modrate-Intensity training-Li, HIT-Li: High-Intensity training+Li

[a] $P < 0.01$ compared to the CTL group,

[b] $P < 0.01$ compared to the LIT-Li group,

[c] $P < 0.001$ compared to the HIT-Li group.

affected the expression of anti-apoptotic gene, leading to cardiac improvement in lithium-treated rats.

Caspase-3, which is the joining point of the apoptotic signaling pathway, may be a common portion of different apoptotic signaling pathways. Once caspase-3 was activated, apoptotic pathway was started [46]. Bax can activate a few little molecules to enter into cytoplasm, coming about in cell apoptosis [11]. Bcl-2 is an anti-apoptotic protein that can compete against Bax to play its anti-apoptotic work [9]. Regular exercise training, with its preconditioning effects, is one of the most effective protective factors against cellular death [31]. In this project, moderate-intensity training can induce its anti-apoptotic effects through improving the expression of the Bcl-2 gene in heart tissue. The Bcl-2 gene plays an important role in cell survival and protects cardiac myocytes from various stressors [25,36]. In parallel with our result, Ghajari et al. showed that eight weeks of moderate-intensity endurance training significantly increased Bcl-2 in the cardiac tissue of mice [12]. Ham O et al. also reported that aerobic training significantly increased anti-apoptotic Bcl-2 gene expression [13]. It seems that mitochondria play a critical role in regulation of apoptosis in cardiac muscle cells. This includes mobilization of pro-apoptotic proteins to the mitochondrial membrane (e.g. Bax) resulting in a release of mitochondrial-housed factors (e.g. cytosolic cytochrome c; Ref.) to the cytosol and initiation of downstream apoptotic signaling [37]. It can be concluded that moderate-intensity training can have preventive effects on apoptotic signaling by ameliorating the mitochondria performance [43], and significantly impact the improvement of cardiac apoptosis by increasing the expression of the anti-apoptotic Bcl-2 gene, and in other words, it prevents cardiac apoptosis due to the use of lithium drugs.

The findings of this research indicate that moderate-intensity training can ameliorate the toxic effects of lithium in cardiac tissue, but high and low-intensity training combined with lithium intake cannot significantly improve cardiac damage caused by lithium. Meanwhile, low-intensity training showed positive effects on the increased expression Bcl-2 gene with lithium consumption. Consistent with the current research, Belardinelli et al. reported that patients with chronic heart failure may achieve a significant improvement in the cardiac function capacity with a low-intensity training regimen, and based on the results, this study can positively affect the expression of anti-apoptotic Bcl-2 genes in the heart. The mechanism responsible for this desirable effect includes an increase in mitochondrial density, which indicates an improvement in the oxidative capacity of skeletal muscles after exercise [45], and training with moderate-intensity can have more effects on the mitochondrial content of skeletal muscles [18]. Interestingly, the effects of training on Bcl-2 may be related to the intensity of training.

Cradiac damage caused by high doses of lithium [2] can occur with doses higher than 1.5 mmol/liter, and more severe damages occur with doses higher than 2.5 mmol/liter [3]. In this regard, Salimi et al. showed that lithium consumption disrupts mitochondrial function, oxidative stress, initiates cytochrome C release, and ultimately leads to cardiac cell death [38]. In this study, since the pre-apoptotic Bax gene had the lowest

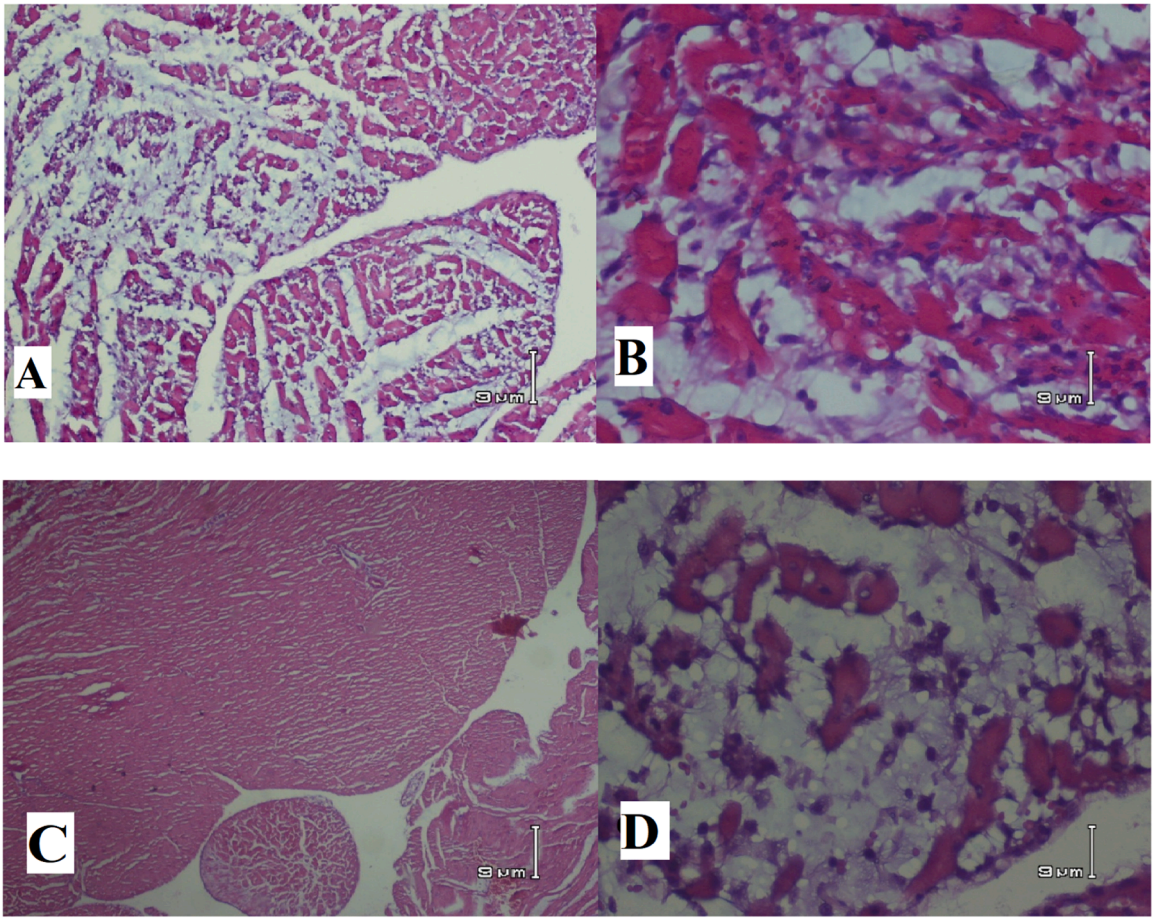


**Fig. 5.** Histopathological examination of heart tissue in various groups. Li (A): Edema, necrosis of muscle fibers in the area below the endocardium and myocardial thickness in severe form; LIT-Li (B): Necrosis of muscle cells, their senescence degeneration, severe intercellular edema and hemorrhage and hyperemia of vessels and infarction of neutrophil cells and macrophages; MIT-Li (C): Mild intermuscular edema without degeneration necrosis with edema changes under the endocardium and the papillary muscle is more and less in the depth of the myocardium; HIT-Li (D): Sens degeneration and necrosis of muscle fibers and the infiltration of defense cells between them widely, along with the withdrawal of defense cells from the vessel wall and entry into the wavy stroma.

expression level in the moderate-intensity training group compared to other groups, it can be concluded that moderate-intensity training has the best performance in improving the heart recovery prevented cardiac apoptosis by reducing the expression of this gene.

Linearly, the expression of the Bax gene in high-intensity training group was higher compared to the moderate-intensity training group. Magrini et al. showed that during high-intensity training, increased ROS levels and oxidative stress, chronic fatigue syndrome (CFS), overtraining syndrome, changes in endocrine function, immune system, systemic inflammation can cause apoptosis in cardiac cells [28]. In this study, high and low-intensity training could not significantly affect the over expression of Bax gene or reduce apoptosis, suggesting that these two exercise intensities do not play an effective role in improving cardiac apoptosis during lithium consumption. Furthermore, the pathology results indicated that moderate-intensity training may have a protective effect on the myocardium against lithium-induced cardiac damage.

Exercise training at low, moderate, and high intensities reduces caspase-3 gene expression following lithium use. Most studies indicate that the level of caspase-3 is reduced by following physical activity [39, 41,42,5] or remain unchanged [20]. Caspase-3 gene expression increases in the heart during lithium drug use, so the role of exercise in reducing gene expression and improving cardiac apoptosis, which is undeniable given the above explanations. From these findings, it can be inferred that while lithium has a negative effect on the caspase-3 gene expression, physical activity at different intensities significantly reduces the negative effects of this drug.

Cardiac troponin I is a regulatory protein of the myocardial contraction system and increased cTnI increases the risk of acute myocardial injury [44]; the present study showed that the highest levels of cTnI were in the Control+Lithium group; From the present research, we found that the serum levels of cardiac troponin I increase up to eightfold following lithium consumption. As studies have shown, cardiac troponin I is a golden indicator of myocardial tissue damage and a specific marker of myocardial damage, and its increase in serum is important in diagnosing myocardial ischemia [4,14]. Moderate-intensity training reduces the release of cTnI from heart tissue and has a protective effect on heart cells. Badawy et al. mentioned that cardiac damage caused by lithium is due to oxidative stress and apoptosis, and this is indicated by a significant increase in cardiac troponin I levels [8]. Legaz et al. also reported that exercise intensity affects cTnI release, and when exercise intensity decreases, a significant effect of exercise intensity on peak cTnI levels after exercise is observed [26]. Based on studies conducted in this area, it can be concluded that moderate-intensity training reduces the pressure on the heart, leading to a reduction in cTnI release. This exercise intensity has had desirable effects on cTnI levels in the current study and, in other words, has significantly it reduced the harmful effects of lithium on the heart of rat. One of the limitations of this study is the lack of high-quality and adequate studies. Therefore, it is not possible to clearly evaluate the optimal exercise-based cardiac rehabilitation protocol. This study highlights the need for high-quality research on moderate and low-intensity exercise in cardiac patients. Furthermore, another

limitation of the current study is that the amount of proteins involved in cell apoptosis was not measured.

## 5. Conclusion

Various studies suggest that different exercise intensities have differing protective effects on the heart. According to the present research, it became evident that among different exercise intensities, the most protective effect against cardiac apoptosis occurred with moderate exercise intensity, which may potentially serve as a therapeutic target for reducing cardiac damage. This study conducted this measurement for the first time and demonstrates that moderate-intensity training has positive effects on reducing lithium toxicity.

## CRediT authorship contribution statement

**Mehdi Abbaspoor:** Writing – review & editing, Writing – original draft, Visualization, Validation, Funding acquisition, Formal analysis, Data curation. **Soheil Aminizadeh:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Methodology, Data curation, Conceptualization. **Shadan Saberi:** Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **Zahrasadat Roholamini:** Writing – review & editing, Writing – original draft, Software, Resources, Project administration, Methodology, Investigation, Data curation, Conceptualization.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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## Data availability

Data will be made available on request.

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