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Protective effect of zinc sulfate and continuous/interval training on heart oxidative stress in morphine-withdrawal syndrome in rats

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Abstract

Opium abuse results in oxidative damage in several tissues such as heart. We investigated the effect of continuous and interval training (CT and IT) with zinc sulfate (ZS) consumption on oxidative stress in the heart in morphine-dependent rats following withdrawal syndrome (WS).
Seventy male Wistar rats were randomly divided into seven groups: Control, WS rats, WS rats receiving ZS 9 mg/kg by gavage, WS rats with CT, WS rats under IT, WS rats with CT and ZS and WS rats with IT and ZS. Animals were addicted to morphine sulfate. Interval and continuous exercises performed five days/weeks by running on a treadmill. Heart tissue and serum samples were collected and markers of oxidative stress were measured by spectrophotometric methods, also creatine kinase (CK-MB) and troponin I (TPI) were measured by spectrophotometric methods.
The results showed that the receiving ZS along with CT/IT caused increased heart catalase activity and decreased the serum malondialdehyde level as compared to the withdrawal group. In addition, IT/ZS showed significantly increased in superoxide dismutase activity in serum and heart tissue as compared to the morphine withdrawing. Furthermore, it is observed that ZS and CT/IT made a significant reduction in CK-MB and TPI levels in comparison to the morphine group. Also, the IT/ZS supplement reduced the serum level of cholesterol, triglycerides, low-density lipoprotein-cholesterol, Very-low-density lipoprotein-cholesterol and Atherogenic Index compared to the withdrawing group.
In conclusion, CT/IT scheduled exercises therapy combined with ZS improves the oxidative stress in morphine-induced heart injury in WS rats.

Key words: Continuous training, Interval training, Morphine, Zinc Sulfate, Heart.

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Introduction

Opium addiction as a commonly prevalent (1-2 % worldwide) predicament has been shown to have adverse effects on various organs in human body (1). Recently, it has been reported that opiate abuse exerts its toxic effect through induction of oxidant-antioxidant imbalance (1). Moreover, some researchers have shown that chronic or acute opium abuse results in oxidative damage in several tissues including liver and heart (2, 3). Considering that the damage caused by opium addiction or withdrawal syndrome is attributed to the formation of oxidative stress, the involvement of heart tissue in opium addicts suggests the involvement of the cardiovascular system in opium addiction. So far, several studies have showed the adverse effects of opium abuse on cardiovascular system and also announced that opium misuse could result in increased morbidity from cardiovascular system disease. Further studies have reported that oxidative stress and inflammatory factors have play a critical role in heart failure and opioid misuse has been suggested as a predisposing factor for heart disease (4, 5). On the contrary, zinc, as a micronutrient with potential antioxidant activity, is reported to be an effective antioxidant in protecting various tissues from damage caused by oxidative stress (6, 7).

Accordingly, exercise training is highly recommended in patients with heart failure (8), however, its effectivity may vary according to various dose parameters, session duration, specifically program length, and frequency and intensity or workload (9). More importantly, Regular resistance exercise has shown to attenuate the oxidative damage (10). In some cases, it has been reported that regular exercise training results in increased antioxidant enzymes activity and then improves body defense against oxidative damage (11). Zinc supplementation along with the exercise have been proposed to have synergistic effects and attenuate the oxidative damage. Recent reports have demonstrated that zinc supplementation can support the cellular repair events during exercise recovery. In addition to providing structural integrity and contribution to the enzymatic activities of metalloenzymes, such as carbonic anhydrase and lactate dehydrogenase, zinc and its transporters play a pivotal role in intracellular signaling pathways such as energy metabolism and oxidative stress (12, 13).

Given that the exercise training ameliorate the oxidative stress in cardiac tissue, zinc and its supplementations have been proposed to coordinate with exercise in heart tissue oxidative damage attenuation. Therefore, the present study was designed first, to investigate the possible oxidative stress induced by withdrawal syndrome on heart tissue and second, to examine the synergistic or antagonizing effects of exercise training and zinc supplementation on heart tissue oxidative stress of opium addicted rats.

Material and Methods

In this experimental study, we used 70 male Wistar rats weighing 250±20 g. The animals were maintained in the animal house with free access to water and food throughout 12hours of daylight and 12-hours of darkness. The study followed the principles of the declaration of Helsinki and was approved by the Medical Ethics Review Board of Jiroft University of Medical Sciences (Ir.jmu.rec.1394.13) (14).

Animals were randomly categorized into seven groups (10 rats each):

- Group 1: Healthy control rats (C)
- Group 2: Withdrawing rats without exercise training and receiving normal saline and usual diets (WS)
- Group 3: Withdrawing rats receiving 9 mg/Kg zinc sulfate orally (WS + ZS)
- Group 4: Withdrawing rats under continuous exercise training (WS + CT)
- Group 5: Withdrawing rats under interval exercise training (WS + IT)
- Group 6: Withdrawing rats under continuous exercise training receiving 9 mg/Kg zinc sulfate (WS + ZS + CT)

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Table 1. Continuous and interval training protocols

Week	Continuous group	Interval group
1	16 (12 m/min)	2 × 8 (12 m/min)
2	20 (12 m/min)	2 × 10 (12 m/min)
3	24 (13 m/min)	2 × 12 (13 m/min)
4	28 (14 m/min)	2 × 14 (14 m/min)
5	33 (15 m/min)	3 × 11 (15 m/min)
6	39 (16 m/min)	3 × 13 (16 m/min)
7	45 (17 m/min)	3 × 15 (17 m/min)
8	51 (18 m/min)	3 × 17 (18 m/min)

- Group 7: Withdrawing rats under interval exercise training receiving 9 mg/Kg zinc sulfate (WS + ZS + IT)

Addiction

Animals in morphine-dependent groups were addicted to 0.4 g/L oral morphine sulfate for 21 days. In addition, sucrose (40 mg/mL) was added to drinking water due to the bitter taste of morphine. To ensure the morphine-induced dependency in the animals, 1-2 rats in each working group received 1 mg/kg naloxone intraperitoneally (Sigma-Aldrich, St. Louis, MO, USA) (15).

Then animals of the training groups performed continuous and interval running exercise training for five days a week. During 8 weeks, 9 mg/dl zinc sulfate was orally consumed in the groups receiving supplementation, five days every week. Timeline of the experimental design is presented in figure 1.

Continuous training

In this protocol, rats were exercised for 8 weeks, 5 days every week (Continuous group). Rats had performed the training with 12 m/min for 16 minutes. During 8 weeks, the training speed and duration were gradually increased and they exercised for 51 minutes with 18 m/min on the treadmill (Table 1).

Interval training

The training was performed in multi-stage phases for interval group and active recovery was considered. The training was followed with 12 m/min with two 8-minute phases, which was increased to three 17-minute phases with 18 m/min (Table 1) (16).

After the end of the study, fasted rats were anesthetized with ketamine (50 mg/kg) and blood samples were collected and stored at –20 °C. Also, the heart tissue was separated from each rat and cleaned with an ice-cold saline solution and frozen in liquid nitrogen immediately after separation and stored at –70°C until analysis.

Measurement of biomarkers of oxidative stress

Malondialdehyde (MDA), as a marker of lipid peroxidation, was determined using Yagi's methods; total antioxidant capacity (TAC) was measured by Benzi and Strain method using ferric reducing antioxidant power assay (FRAP) (17).

Serum antioxidant enzyme activity assay

Superoxide dismutase (SOD) and catalase (CAT) activity in the heart homogenate and serum were determined using a spectrophotometric method (ZellBio GmbH, Ulm, Germany).

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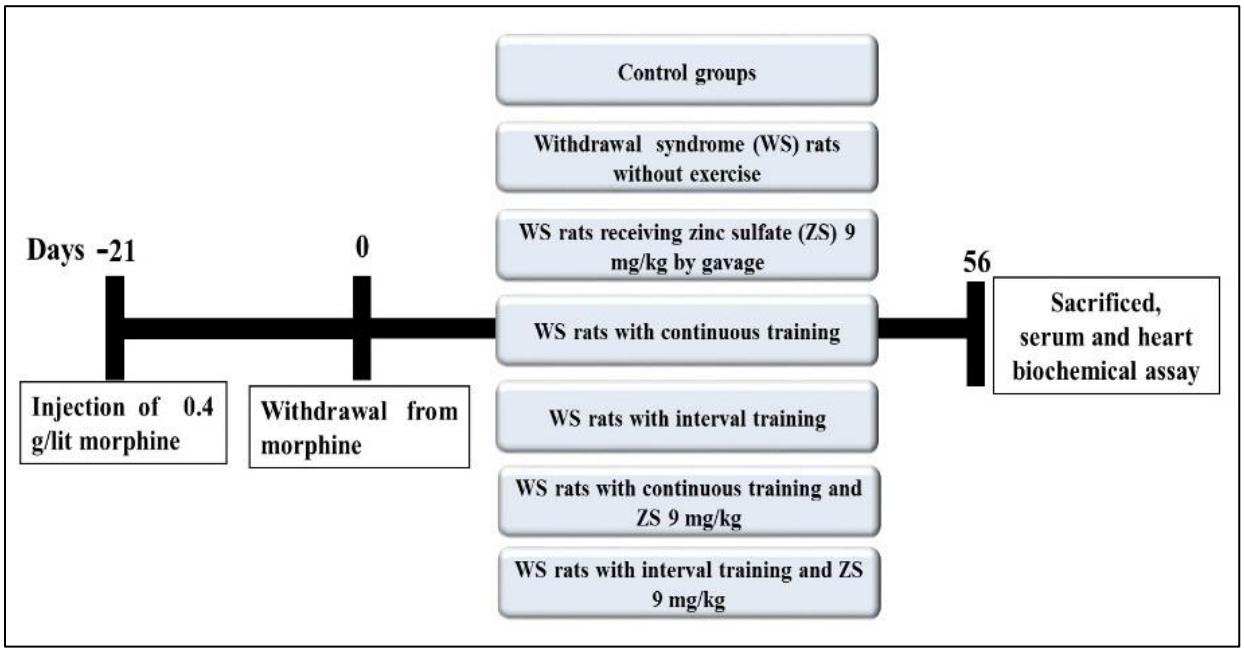

Figure 1. Timeline of the experimental design.

Serum cardiac marker assay

The activities of serum creatine kinase (Total and MB) and troponin I (TPI) were determined using Pars Azmoon kits (Pars Azmoon, Iran).

Measurement of serum biochemical parameters

Serum total cholesterol (Cho), triglycerides (TG) and HDL-C were measured according to the routine procedure using commercially available kits (Pars Azmoon, Iran). Serum low-density lipoprotein-cholesterol (LDL-C) level was calculated from the Friedewald formula (LDL-C = TC - [HDL-C + TG/5]) while serum Very-low-density lipoprotein-cholesterol (VLDL-C) concentration was calculated according to the Nobert formula (VLDL-C = TG/5). The atherogenic Index (AI) was calculated as a ratio of TG/HDL-C (18).

Statistical Analysis

The results were expressed as mean ± SD. Data were analyzed by SPSS version 16, and Prism 6.0 softwares (GraphPad, San Diego, CA, USA). Values of $p > 0.05$ were considered non-significantly different, while those of $p < 0.05$ were considered significantly different.

Results and Discussion

The results of serum lipid peroxidation level

Opium abuse or withdrawal syndrome is one of the main problems in various countries. Opium use in cardiac patients has shown to be an important medical problem in addiction (19). It is believed that opium consumption may have beneficial effects on cardiac diseases, as well as opium consumption reported to be substantially higher in cardiac patients than general population (19). However, the valuable data from prospective cohort studies have suggested that opium consumption may resulti in increased death risk in patients with circulatory disease (20). These confusing reports in various study prompted us to investigate the opium adverse effects on heart tissue in withdrawal syndrome as well as the possible protective effects of exercise training along with zinc sulfate supplementation on withdrawing rat's heart tissue.

Our findings demonstrated that serum malondialdehyde (MDA) level considerably increased in rats with withdrawal syndrome; further investigations showed that exercise

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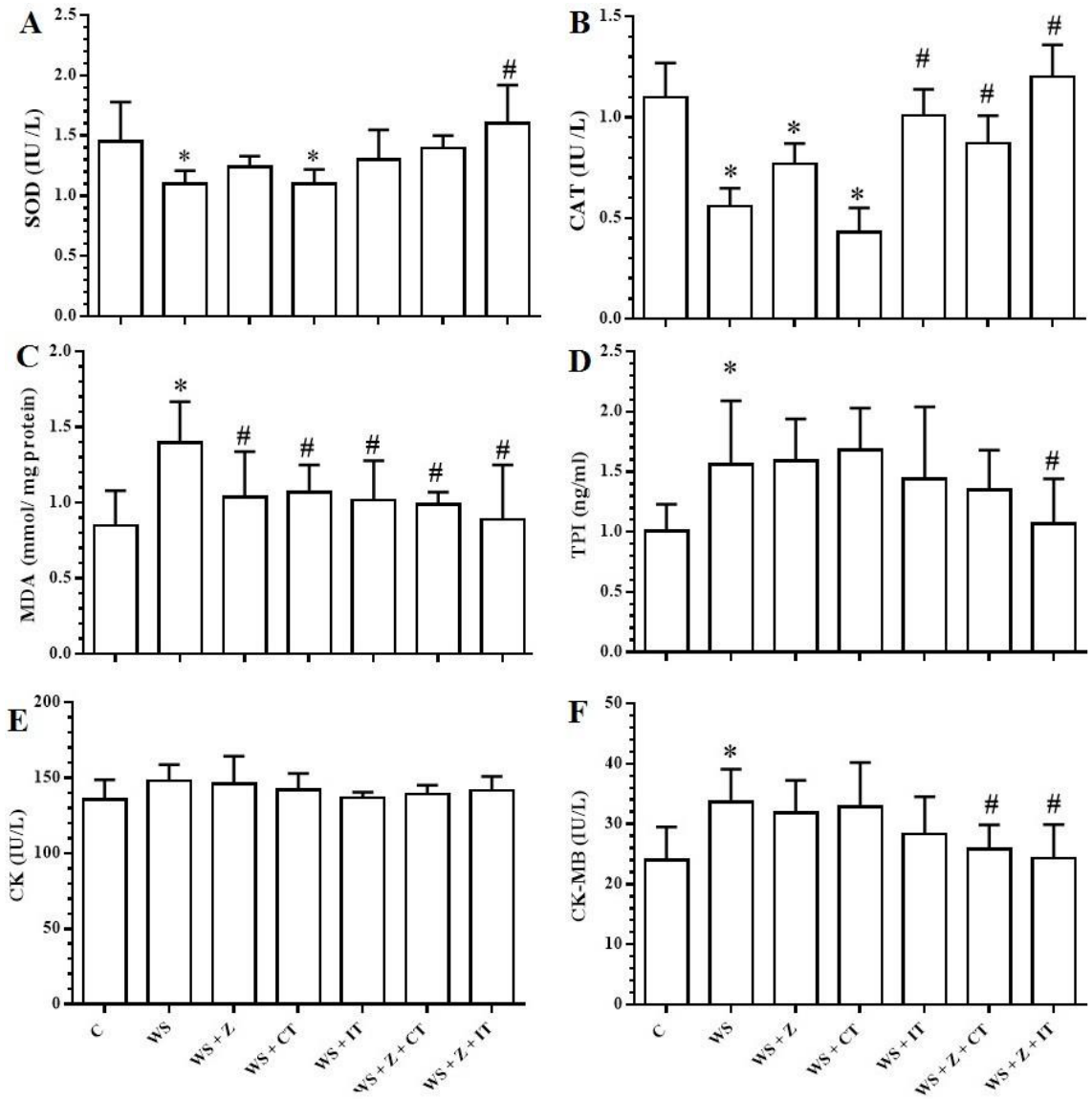

Figure 2. Level of serum (A) superoxide dismutase activity (B) catalase activity (C) malondialdehyde (D) Troponin I (E) creatine kinase total and (F) creatine kinase- MB after eight weeks of treatments. Results were expressed as mean ± SD. (C) Control group, (WS) withdrawal syndrome, (Z) Zinc sulfate, (CT) continuous training, (IT) interval training. * Significant differences compare with the control group, # significant difference compare with the WS group (p <0.05).

training in both CT and IT schedules result in significant decreased serum MDA levels in withdrawing rats (p<0.05). Moreover, using zinc sulfate supplementation improved lipid peroxidation level when administered with CT and IT exercise training schedules compare thane alone administration (Figure 2C). Reactive oxygen species (ROS) were generated during metabolic processes in various cells. Recently, various studies have shown that morphine addiction is directly able to induce ROS generation in different cells (21). Results of present study demonstrate that administration of morphine and following withdrawal from morphine resulted in development of oxidative stress in heart tissue. Oxidative induction was

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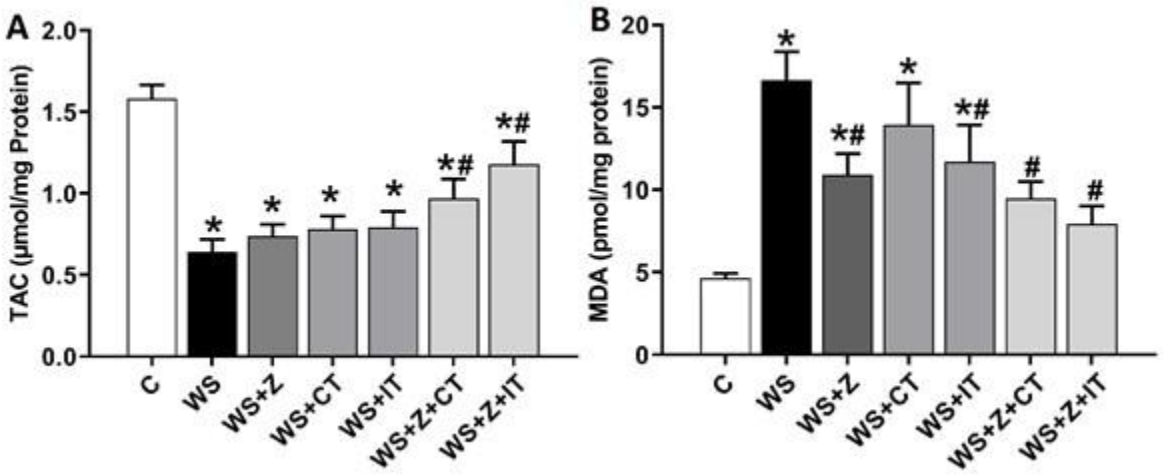

Figure 3. Level of heart (A) Total Antioxidant Capacity (B) Malondialdehyde after eight weeks of treatments. Results were expressed as mean ± SD. (C) Control group, (WS) withdrawal syndrome, (Z) Zinc sulfate, (CT) continuous training, (IT) interval training. * Significant differences compare with the control group, # significant difference compare with the WS group (p <0.05).

indicated by significant increase in heart tissue and serum MDA levels and a progressive decrease in heart tissue TAC level in rats with morphine withdrawal.

The result of serum antioxidant enzymes activity

As shown in figure 2A, serum SOD activity was significantly decreased in withdrawal syndrome group as compared to the control group, while higher SOD activity was seen in rats which received zinc sulfate along with IT exercise training ($p<0.05$). Rats with withdrawal syndrome showed markedly lower serum CAT activity when compared to the control rats (figure 2B). However, IT exercise training led to significant increase in CAT activity, and using zinc sulfate supplementation with IT training was more effective on serum CAT activity ($p<0.05$). Additionally, CT exercise training along with zinc sulfate results in elevated serum CAT activity ($p<0.05$).

Moreover, our investigations revealed that SOD and CAT enzymes activity markedly decreased in heart tissue and serum of rats with withdrawing from morphine. However, zinc sulfate supplementation was found to be capable of attenuating oxidative stress, especially when administrated along with exercise training. Zinc sulfate co-administration with IT and CT scheduled exercise strongly attenuated the lipid peroxidation in heart tissue of morphine-withdrawing rats. Our findings indicated that serum and heart tissue CAT activity levels were markedly elevated in withdrawing rats which received zinc sulfate and exercise in both IT and CT scheduled exercise. Furthermore, the increase in heart tissue and serum SOD activity was induced by IT planned exercise along with zinc sulfate. In agreement with our findings, Abdel-Zaher and colleagues have reported that repeated administration of morphine and withdrawing from morphine resulted in development of oxidative stress (22). Substantial evidences in previous studies suggest that a metabolite of morphine could directly resulted in superoxide radical formation (23). In line with our data, another research demonstrated that morphine was able to metabolize into free radicals and leads to oxidative damage in various tissue via over generation of ROS (24). On the other hand, exercise training had beneficial effects on the activity of antioxidant enzymes such as CAT and

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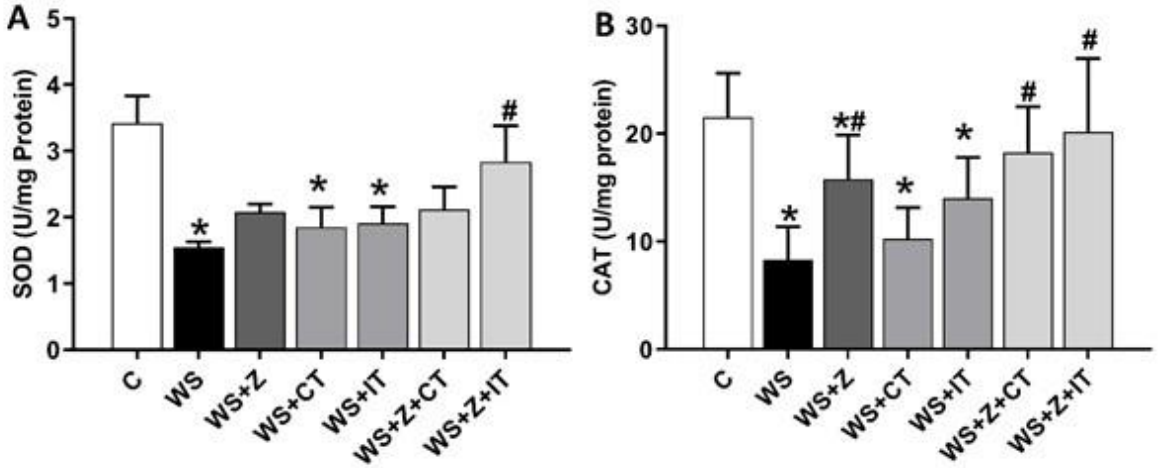

Fig 4. Level of heart (A) Catalase activity (B) Superoxide Dismutase activity after eight weeks of treatments. Results were expressed as mean ± SD. (C) Control group, (WS) withdrawal syndrome, (Z) Zinc sulfate, (CT) continuous training, (IT) interval training. * Significant differences compare with the control group, # significant difference compare with the WS group (p <0.05).

SOD, which suggest that exercise training may be increased the mitochondrial content and function (25). Our findings are consistent with the literatures indicating the endurance training but not exhausting and acute training up-regulated the activity of the antioxidant enzymes and alleviates the oxidative damage (26, 27). There is also substantial evidence that exercise training suppressed myocardial tissue oxidative damage in animal models (28, 29). However, further investigations revealed that IT exercise was superior to CT exercise in alleviating oxidative stress in heart tissue of withdrawing rats. Previous studies have suggested that IT exercise was more effective than CT exercise on oxidative stress attenuation (30, 31).

The results of serum cardiac markers

The activity of Creatine Kinase MB (CK-MB) and the troponin I (TPI) levels as cardiac-specific markers were markedly higher in the serum of rats with withdrawal syndrome ($p<0.05$). Although the exercise training scheduled in CT and IT resulted in decrease serum CK-MB activity, the difference between withdrawal syndrome group and CT and IT groups were statistically significant ($p<0.05$) (figure 2F). Our results also showed a significant decrease in serum TPI content of withdrawing rats which received zinc sulfate along with IT exercise training ($p<0.05$) compared with withdrawal syndrome group (figure 2D). Serum total CK activity measurement showed no significant difference between study groups ($p>0.05$) (figure 2, E).

Despite the no changes in serum total CPK activity, we have found an increase in serum CK-MB and TPI levels of withdrawing rats, suggesting that withdrawal syndrome may contribute to cardiac myocytes membrane disruption. CK-MB and TPI as the cytosolic enzymes are the main sensitive markers in detecting of myocardial cell injury during and following WS caused oxidative stress (32). These markers leak out from damaged cardiac myocytes when the cell membrane becomes permeable or ruptures. According to the evidences, WS induced oxidative stress may contribute to injury in myocytes. However, using antioxidant agents such as zinc sulfate along with IT scheduled exercise significantly suppressed oxidative stress-caused damage and also decreased TPI levels. We also found that zinc sulfate

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supplementation co-administered with IT and CT scheduled exercise had protective effects on WS following oxidative stress-caused damage in cardiac myocytes and also resulted in markedly decrease in CK-MB level.

Biomarkers of oxidative stress in heart tissue

Figure 3 shows the TAC and MDA levels of heart tissue in study groups. Our findings showed a significantly lower TAC level in withdrawing rats as compared to control group. Using zinc sulfate with both CT and IT exercise training schedules resulted in significant increase in heart TAC level when compared to withdrawing group ($p<0.05$), but zinc supplementation with exercise could not bring total antioxidants to normal level ($p<0.05$). The MDA concentration as a potential index for lipid peroxidation of heart tissue was significantly elevated in withdrawal syndrome group ($p<0.05$). On the other hand, it was observed that the heart tissue MDA concentration was markedly decreased in withdrawing rats which received zinc sulfate and IT exercise ($p<0.05$). Moreover, zinc sulfate along with exercise training in CT/IT planning decreased the heart tissue lipid peroxidation level as compared to the withdrawal group ($p<0.05$). Administration of zinc sulfate supplementation alone and IT alone reduced the MDA level in heart tissue and restored the MDA levels but could not bring MDA to the range of healthy rats ($p<0.05$).

Oxidative stress-induced damage in myocardial cells depends on the imbalance between cells antioxidant defense and excess of ROS production (33). In the present study, over produced the MDA level and decreased TAC level in heart as well as decreased antioxidant enzymes activity such as SOD and CAT are the main indicators of oxidative stress. In this regard, recent researches have suggested that regular exercise training promotes attenuation of oxidative damage in various tissues in addicted patients (29, 34, 35). Of interest, serum and heart tissue level of MDA were markedly decreased during exercise. In the present study, we also found that zinc sulfate supplementation combined with IT or CT exercises can further improve heart tissue oxidative stress in morphine withdrawing rats. Zinc supplementations act as effective antioxidant (36), which is consistent with present study results, in which the oxidative stress markers were improved using zinc sulfate combined with IT or CT exercises. This combined therapy was more effective especially when administrated along with IT scheduled exercise.

Status of antioxidant enzymes in heart tissue

As illustrated in figure 4, rats with withdrawal syndrome showed a significant decrease in SOD activity as compared with control group ($p<0.05$). Although, zinc administration and exercise training scheduled in CT and IT improved SOD activity in withdrawing rats, only zinc sulfate co-administration with IT exercise training resulted in marked increase in SOD activity as compared with withdrawal syndrome rats ($p<0.05$). Our investigations revealed that CAT activity as an antioxidant enzyme significantly decreased in withdrawal syndrome group when compared to control group ($p<0.05$). However, zinc sulfate alone and in combination with CT/IT exercise training administration considerably enhanced the CAT activity in withdrawing rats in compression to control rats ($p<0.05$).

Serum biochemical parameters

Table 2 shows the results of cholesterol, triglyceride, HDL-C, LDL-C and AI in study groups. Our investigations demonstrated that serum cholesterol, cholesterol, triglyceride, LDL-C and AI levels were markedly increased in withdrawing rats, while the results revealed that zinc sulfate co-administrated with IT improve lipid profiles in comparison with withdrawing control group ($P<0.05$).

The present experimental study showed that opium abuse and withdrawal syndrome significantly increased the oxidative stress marker in serum and heart tissue. Our investigations showed a derangement in serum lipid profile. Withdrawing rats exhibited a higher serum Cho, TG, VLDL, LDL-C, and AI as

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Table 2: Serum biochemical parameters in different studied groups after eight weeks of treatments.

Parameter/ Group	Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL-C (mg/dl)	VLDL-C (mg/dl)	LDL-C (mg/dl)	AI (TG/HDL-C)
C	92.00±4.8	97.33±7.50	36.33±2.16	17.46±1.50	38.60±5.02	2.63±0.10
WS	119.91±9.54*	156.84±12.58*	31.09±1.97	31.368±2.85*	57.45±6.24*	5.04±0.2*
WS + Z	109.37±5.29	147.52±15.98	30.87±2.65	29.504±2.01	48.99±4.89	4.77±0.12
WS + CT	106.29±8.19	112.81±10.27	32.89±3.57	22.562±1.65	50.83±5.4	3.42±0.14
WS +IT	100.75±7.16	113.17±8.2	33.97±1.25	22.634±1.49	44.14±3.89	3.33±0.21
WS + Z + CT	101.28±4.21	108.02±11.38	34.09±2.38	21.604±2.08	45.58±5.14	3.16±0.19
WS + Z + IT	95.71±6.98#	96.18±6.26#	35.84±1.95	19.236±2.15#	40.63±6.01*#	2.68±0.17#

Results were expressed as mean ± SD. (C) Control group, (WS) withdrawal syndrome, (Z) Zinc sulfate, (CT) continuous training, (IT) interval training, (AI) Atherogenic Index. * Significant differences compare with the control group, # significant difference compare with the WS group ($p < 0.05$).

compared to control rats. However, further investigations demonstrated that co-administration of zinc sulfate supplementation with IT scheduled exercise appeared to be the most effective on opium-caused dyslipidemia in withdrawing rats. In line with our findings, various studies demonstrated that opium may aggravate dyslipidemia in addicted patients and resulte in elevated TG, Cho and LDL-C as well as decreased the HDL-C (16, 37, 38). Opioids lipolytic effects suggested to be the possible mechanism for dyslipidemia and increased serum value of TG, Cho and LDL-C in addicted patients (38); hypercholesterolemia induced by opium consumption is the main risk factor for atherosclerosis and cardiac damages (16). Moreover, recent reports have shown that application of opioid antagonists strongly prevents hypercholesterolemia and HDL-C decrement in opium administrated rats (38, 39).

Conclusion

In conclusion, this study, by showing that morphine withdrawing following addiction adversely affects the heart tissue via inducing oxidative stress in myocardial cells, suggest that the zinc sulfate supplementation and exercise combined therapy may contributes to heart tissue oxidative damage attenuation during morphine withdrawing. In comparison to CT exercise, IT exercise was more effective on heart tissue when performed along with zinc sulfate supplementation. Collectively, the results of this study suggest that exercise training and zinc sulfate combined therapy alleviates oxidative damage induced by morphine abuse.

Ethical approval

All ethical considerations were confirmed by the given instructions of Hamadan University of Medical Sciences (IR.UMSHA.REC.1395.195).

Authors' Contribution

Nejat Kheiripour and Reza Mirzaei designed the experiments and wrote the manuscript; Sahar Hassani, Davod Ilbeigi, Hadi Ghasemi and Hassan Ghasemi performed the experiments; Reza Ghahremani, and Ali Heidarian Poor contributed in performing the experiments and analyzed the results.

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Declaration of Competing Interest

The author declares that they have no known Conflict of Interest or personal relationships which could have influenced the information reported in this manuscript.

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