



Effects of Resistance, Endurance, and Concurrent Exercise on Oxidative Stress Markers and the Histological Changes of Intestine After Morphine Withdrawal in Rats

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Abstract

Objectives: The aim of this study was to evaluate the effects of resistance, endurance, and concurrent exercise on oxidative stress markers and histological changes of the intestine after morphine withdrawal in rats.

Methods: A total of 30 male Wistar rats were randomly divided into 5 groups (n=6) including healthy control, withdrawal (rat received morphine for 21 days and 8 weeks of withdrawal period), withdrawal + endurance exercises, withdrawal + resistance exercises, and withdrawal + concurrent exercises. The rats practiced endurance, resistance, and concurrent exercises for 10 weeks. Then, their intestines were removed and used for biochemical and histological analysis. Next, several factors were measured such as total protein levels, malondialdehyde (MDA), reduced glutathione (GSH), total antioxidant capacity (TAC), and total oxidant status (TOS). Finally, the morphological alteration of intestine was examined under the light microscope.

Results: Morphine withdrawal significantly increased the levels of MDA in the intestine of withdrawal rats compared to those of the control group while endurance, resistance, and concurrent exercise reduced the MDA levels in the intestine. In addition, morphine withdrawal led to a decrease in TAC and GSH levels in the intestine compared to control rats whereas endurance, resistance, and concurrent exercise noticeably increased TAC and GSH levels. Interestingly, the change in the concurrent group was more significant. Moreover, the levels of TOS demonstrated a significant increase in the addicted rat as compared to the control group. Conversely, endurance, resistance, and concurrent exercise significantly decreased TOS levels and the reduction was more significant in the concurrent group. Finally, the intestine of withdrawal rat was morphologically abnormal while it restored by the exercise.

Conclusion: Overall, endurance, resistance, and concurrent exercise significantly normalized oxidative stress and the morphological changes of the intestine in withdrawal rats.

Keywords: Morphine, Reduced glutathione, Rat, Oxidative stress, Intestine

Received: 19 May 2018
Accepted: 2 November 2018
ePublished: 23 November 2018



Background

Opium is extracted from a plant called "*Papaver somniferum*" (1) and many people use this agent for recreational or medical purposes worldwide, especially in different Asian countries (2). According to previous evidence, more than 20 million people are addicted to opium (3) that acts as an analgesic, anticough, and antidiarrheal agent (2). Previous reports indicate that patients with chronic disorders such as coronary artery disease, hypertension, and hyperglycemia use more opium than healthy people due to traditional beliefs (4). In some countries in Asia, about 10% of patients with a history of myocardial infarction consume opium and believe that it can reduce blood lipid, pressure,

and glucose levels (5).

In addition, opium contains about 50 types of alkaloids, various sugars, several organic acids, and water. Alkaloids are divided into two main groups including benzyloisoquinolines and phenanthrenes, along with numerous minor groups (5). Phenanthrenes encompass codeine, thebaine, and morphine and constitute major psychoactive ingredients. Morphine is the most copious alkaloid and active compound which leads to addiction (6). Experiments conducted on mice reveal that both morphine withdrawal and chronic morphine administration can reduce host defence to enteric bacteria (e.g., *Pseudomonas aeruginosa* and *Salmonella enterica*) stimulate spontaneous

sepsis in animal models, and sensitize mice, induced by *Acinetobacter baumannii* infection or lipopolysaccharide (LPS), to mortality (7, 8). In fact, morphine withdrawal and chronic morphine administration increase epithelial barrier dysfunction and consequent bacterial translocation from the small intestine, increase serum interleukin-6 levels and induce the progression of LPS-induced inflammation (9,10). Meng et al reported that morphine disrupts intestinal epithelial barrier and thus increases bacteria translocation by toll-like receptor (TLR) signaling. It was also shown that morphine injured intestinal epithelium and increased inflammatory cell infiltration in the villi of the mice. Further, they found that morphine increased TLR2 and TLR4 in the small intestine (11). Furthermore, animal studies demonstrated that opium leads to morphological changes in the small intestine and increase oxidative stress (1). Opium can also cause ulceration and fibrosis in the small intestinal of opioid-dependent patients (12).

Several experiments confirmed that exercise has useful effects on biochemical factors and total withdrawal score in morphine withdrawal animals (13, 14). Similarly, blood exercise was reported to normalize cortisol and glucose levels in morphine withdrawal rats (13). Moreover, moderate exercise can ameliorate the function of the immune system in morphine withdrawal (15). Likewise, it was approved that opium aggravates inflammatory factors in the blood. In this respect, Asgary et al showed that inflammatory markers including nitric oxide, some complementary factors, and high-sensitivity C-reactive protein were higher in addicted subjects compared to healthy people (16). Based on the findings of another research, opium also increases reactive oxygen species while decreasing the antioxidant capacity in the blood (17). It is further reported that opium-addicted people have a low to moderate grade of inflammation (17). On the other hand, it is well-recognized that exercise can decrease inflammatory markers, normalize lipid profile, insulin sensitivity, and antioxidant activity, and reduce endothelial dysfunction (18). Additionally, regular exercise improves antioxidant enzyme activity while it reduces lipid peroxidation markers (18). It is also reported that physically active elderly subjects display antioxidant enzyme activity and lipid peroxidation concentration similar to young sedentary people, highlighting the importance of regular exercise in reducing the aging-associated injury (18). However, there is little information regarding the effect of exercise on intestinal glutathione (GSH), malondialdehyde (MDA), total antioxidant capacity (TAC), total oxidant

status (TOS), and morphological alteration in morphine withdrawal rats. Hence, investigating the effect of exercise on oxidative alteration in the intestine in opium users could be considerably beneficial for withdrawing the opium. Therefore, in the current, the levels of GSH, MDA, TAC, TOS, and the morphological alteration of intestine were determined in morphine withdrawal rats.

Methods

In this study, 30 male Wistar rats weighing 200-220 g were randomly classified into 5 groups (n=6) and kept under standard conditions with a temperature of 20-23°C and 12:12 hours light/dark cycle having free access to water and food (19). The experimental groups included healthy control, withdrawal (rat received morphine for 21 days and 8 weeks of withdrawal period), withdrawal group + endurance exercises, withdrawal group + resistance exercises, and withdrawal group + concurrent exercise groups.

Addiction

Oral administration was used to induce morphine dependence. Morphine with serial concentrations of 0.1, 0.2, 0.3 mg/mL was orally administrated every 48 hours, then, 0.4 mg/mL was added to water for the rest of the study (21 days). In addition, sucrose (40 mg/mL) was added to drinking water due to the bitter taste of morphine. To ensure that the morphine-induced dependency in the animals, 1-2 rats in each working group received 1 mg/kg naloxone (Sigma-Aldrich, St. Louis, MO, USA, ip.). After naloxone injection, the animals were taken to the isolated room with moderate illumination, and their behaviour was checked for 30 minutes and scored using a modified version of the Gellet-Holtzman scale. Naloxone induced the withdrawal signs of morphine in addicted animals. Then, the withdrawal scores were checked and the exercise process was done (20). All procedures of this experiment were approved by Hamadan University of Medical Sciences (under the ethics code of IR.UMSHA.REC.1395.240).

Endurance Exercises

Rats practiced 5 days a week for 10 weeks and a motorized treadmill was applied for this exercise, according to a previously published method (20), displayed in Figure 1.

Strength Exercise

The animals in the strength exercise group underwent a 5-day/week exercise for 10 weeks and climbed a one-meter

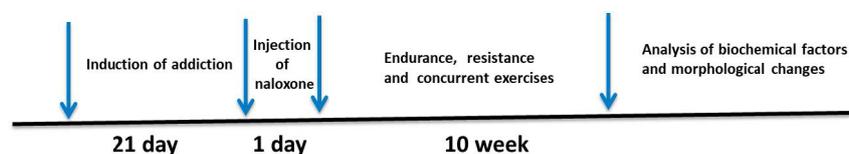


Figure 1. Experimental Timeline.

ladder with an 85° incline 12 times in each period (20).

Concurrent Exercise

Similarly, those in the concurrent exercise group (combined aerobic-resistance) performed half of the strength exercise and endurance exercises on each day (20).

Intestine Homogenate

Small intestine (jejunum) was rinsed with phosphate buffered saline, crushed in liquid nitrogen, and then suspended in lysis buffer containing protease inhibitor cocktail. Next, the intestine homogenate was centrifuged at 20000×g for 15 minutes at 4°C and the supernatant was used for antioxidant tests.

Total Protein Levels

Total protein concentration in tissue homogenates was assessed by Bradford assay using bovine serum albumin as the standard.

Determination of Malondialdehyde Levels

The MDA concentration, as a marker of lipid peroxidation, was measured by a spectrofluorometric method based on the reaction of thiobarbituric acid with MDA. Finally, data were expressed as nmol/mg protein (21, 22).

Determination of Total Antioxidant Capacity and Total Oxidant Status

TAC in the intestine was estimated utilizing ferric reducing antioxidant power assay according to previous studies. The results were presented as nmol/mg of protein. Further, the TOS of intestine was measured applying xylene orange base on the oxidation of ferrous ion to ferric ion in the presence of oxidant agents and the obtained data were provided as nmol/mg of protein (21, 22).

Determination of Glutathione Levels

The reduced GSH concentration was determined by a spectrofluorometric method according to the manufacturer's protocol (ZellBio, Germany).

Histopathological Evaluation

The removed intestine tissue was immersed in 4% formaldehyde at room temperature for 72 hours and then embedded in paraffin and cut into 5 µm thickness. The tissue section was stained with hematoxylin-eosin (H&E method) and examined under the light microscope.

Statistical Analysis

Statistical analysis was performed by using the SPSS software, version 16 (SPSS Inc., Chicago, USA) and data were expressed as mean ± SD. The statistical significance was defined as $P < 0.05$ as well. Finally, the one-way analysis of variance and Tukey multiple comparison tests were used for data analysis.

Results

Biochemical Results

As shown in Figure 2, withdrawal morphine remarkably increased the level of MDA in the intestine compared to the control rats ($P < 0.001$). Conversely, endurance, resistance, and concurrent exercise markedly inhibited the MDA increase in the intestine ($P < 0.001$). On the other hand, the levels of TOS significantly increased in the withdrawal rat as compared to the control group. However, endurance, resistance, and concurrent exercise significantly reduced TOS levels and the decrease was more significant in the concurrent group (Figure 3). Furthermore, morphine withdrawal reduced TAC and the GSH level in intestine tissue compared with the control rats. Eventually, endurance, resistance, and concurrent exercise significantly increased TAC while reducing GSH levels (Figures 4 and 5). Interestingly, the change in the concurrent group was more significant.

Histological Observation

Figure 6 illustrates the normal morphological view of the intestine tissue (H&E 400X). The control rats showed well-preserved cellular integrity and there was no sign of cellular necrosis, degeneration, hemorrhage, or other morphological alterations. However, hemorrhage, degeneration, mononuclear inflammatory cell infiltration, and dilated congested capillaries were found in the

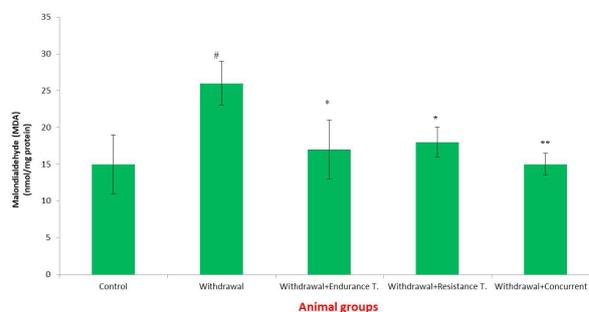


Figure 2. The Effect of Resistance, Endurance, and Concurrent Exercise on the Malondialdehyde Level of the Intestine in Different Treated Rats (n=6). Note. Data are expressed as means ± SD. T: Training. * $P < 0.05$ and ** $P < 0.01$ compared to the withdrawal group, * $P < 0.05$ compared to the control group.

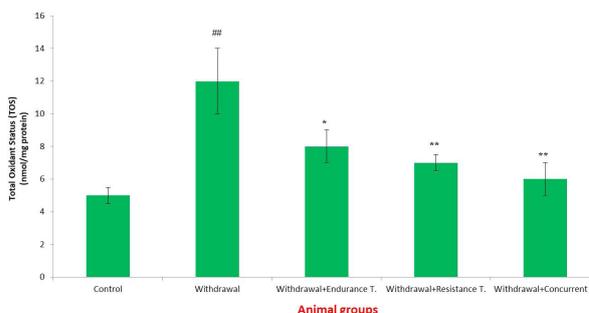


Figure 3. The Effect of Resistance, Endurance, and Concurrent Exercise on the Total Oxidant Status of the Intestine in Different Treated Rats (n=6). Note. Data are expressed as means ± SD. T: Training. * $P < 0.05$ and ** $P < 0.01$ compared to the withdrawal group, * $P < 0.05$ compared to the control group.

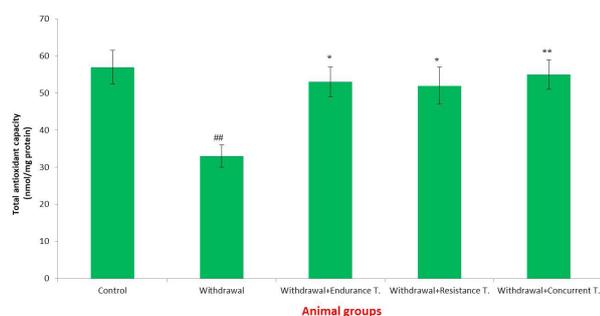


Figure 4. The Effect of Resistance, Endurance, and Concurrent Exercise on the Total Antioxidant Capacity of the Intestine in Different Treated Rats (n=6). Note. Data are expressed as means \pm SD. T: Training. * P <0.05 and ** P <0.01 compared to the withdrawal group, $^{\#}$ P <0.05 compared to the control group.

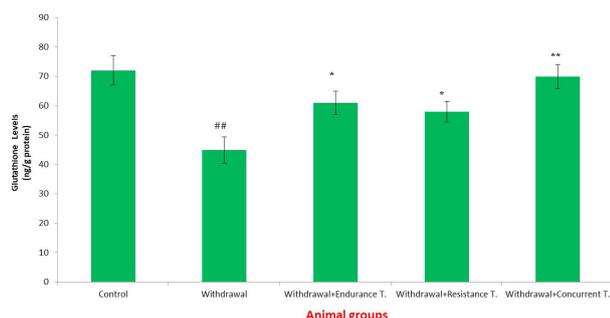


Figure 5. The Effect of Resistance, Endurance, and Concurrent Exercise on the Glutathione of the Intestine in Different Treated Rats (n=6). Note. Data are expressed as means \pm SD. T: Training. * P <0.05 and ** P <0.01 compared to the withdrawal group, $^{\#}$ P <0.05 compared to the control group.

jejunum tissue of addicted group. In all exercise groups, these changes were significantly normalized when viewed under a light microscope.

Discussion

Opium dependency is accompanied by various health problems and subjects with morphine addiction are commonly homeless, unemployed, and use poor diet and low vitamin. This condition influences their gastrointestinal tract and disturbs the function and structure of this organ. Individuals consume different drugs such as methadone for opiate dependence treatment. However, methadone has numerous side-effects and can lead to addiction as well (23). Regular exercise is a useful strategy for opiate addiction programs and has numerous mental and physical health effects (23, 24).

Moreover, regular exercise stimulates body function and health. Both human and animal experiments reported that regular physical activity promotes recovery from all types of disorders (24). Regular exercise also changes the central noradrenergic, dopaminergic, and serotonergic systems (14). Recent data have revealed that endorphin concentrations, chiefly beta-endorphin, increase by regular physical activity. Additionally, regular exercise can increase catecholamines production in the body (when is decreased by morphine withdrawal) thus it can be beneficial in helping the body to recover from morphine addiction and opium withdrawal (14). Likewise, regular exercise is supposed

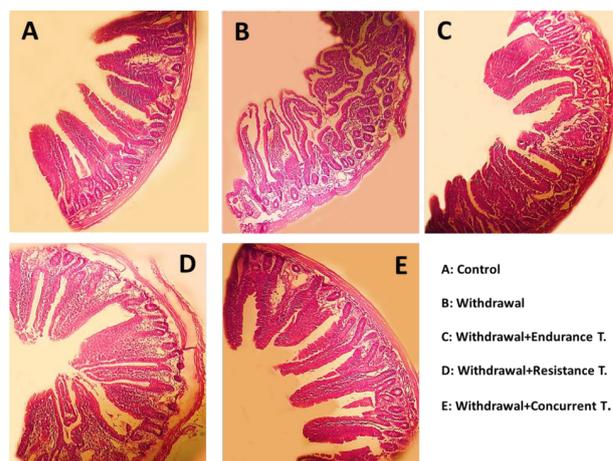


Figure 6. The Histopathological Changes of Intestine Stained With H&E (\times 400) in Different Treated Rats (n=6)

Note. Data are expressed as means \pm SD. T: Training. Control group represented well-preserved cellular integrity and no sign of cellular necrosis, degeneration, hemorrhage, or other morphological alterations was detected as well. However, hemorrhage, degeneration, mononuclear inflammatory cell infiltration, and dilated congested capillaries were detected in the jejunum tissue of the addicted group. These changes were significantly normalized in all exercise groups when viewed under a light microscope.

to enhance and maintain body health, relieve anxiety, and stimulate endogenous opioid release like endorphins within about 30 minutes. The present experiment revealed that oxidative stress markers significantly increased in morphine withdrawal compared with the control while they alleviated by doing exercise.

Based on the findings of the study, endurance, resistance, and concurrent exercise significantly reduced oxidative stress in addicted rats while addiction to morphine significantly increased the oxidative stress in the intestine. Morphine is a strong analgesic opioid which is widely applied for pain reduction in diverse clinical and pathologies condition (e.g., emergencies, cancer, trauma, and assisted ventilation, and the like). High use of this agent can lead to dependence and tolerance (25) and numerous experiments concluded that chronic and acute exposure to morphine can reduce total antioxidant and GSH levels in animal and human liver and brain (26). It was also reported that morphine addiction changes the activity of endogenous antioxidant enzymes such as catalase, glutathione peroxidase, and superoxide dismutase (26).

Our result showed that GSH levels significantly reduced in addicted animals when compared to the control, which is in line with the results of Sumathi et al which demonstrated a significant reduction in GSH concentration in the brain of morphine-addicted animals (27).

In the current experiment, the effects of exercise on the TOS, MDA, and TAC of jejunum were examined in withdrawal animals. Our results confirmed that the levels of TOS and MDA were higher in the withdrawal rat compared to the exercise groups whereas the TAC level was lower in the withdrawal rat in comparison with

exercise groups. MDA and TOS concentrations in the cells are documented as the indicators of oxidative stress. Our finding showed that exercise ameliorated oxidative stress in the intestine of the rats. It was further found that exercise increases antioxidant enzyme activity (24). On the other hand, based on the report of another research, morphine-induced oxidative stress in the body (26). Circu and Aw et al documented that an increase in oxidative stress can lead to mucosal ulcers (28). The balance of antioxidant/oxidative stress is preserved by changing the lifestyle (24). In this study, the GSH concentrations were found to be lower in the intestine of the withdrawal rat compared to the exercise groups. Similarly, Ji et al. found that exercise elevated GSH concentrations in the body (29). Likewise, the increase of oxidative stress markers was accompanied by the morphological alteration of the intestine. In addition, intestine cellular necrosis, degeneration, hemorrhage, and other morphological alterations were observed in withdrawal rats. These alterations were significantly normalized by exercise.

Opium is potentially recognized as a source of free radicals. Further, opium and its metabolite such as morphine increase the oxidative stress by the direct delivery of reactive oxygen species (ROS) and the stimulation of the endogenous ROS generation through stimulating the inflammatory cells (30). Furthermore, morphine can directly decrease the antioxidative defense activity in the body. It appears that ROS formation is one of the main mechanisms behind morphine abuse that is related to a decline in antioxidant enzymes including catalase, glutathione peroxidase, and superoxide dismutase (31). Morphine also induces the release of proinflammatory markers (e.g., TNF- α), and consequently, can cause tissue injury.

In summary, our experiment demonstrated that morphine withdrawal increased TOS and MDA levels in small intestinal while it reduced TAC and GSH levels in the intestine compared to the control rats, which probably could induce the disruption of tight junctions, increases intestinal permeability, and resulted in increased inflammation in the intestine. On the other hand, endurance, resistance, and concurrent exercise increased TAC and GSH levels and normalized morphological changes and the change was more significant in the concurrent group.

Authors' Contributions

IS, FM, EZ and KR prepared the draft of manuscript, and organized oxidative stress tests. EAO planned the study. SSA interpreted the morphological analysis. EAO, EZ and KR performed animal studies and biochemical factors.

Conflict of Interest Disclosures

The authors declare no potential conflicts of interest relevant to this article.

Acknowledgements

This research was supported by a grant from Hamadan University of Medical Sciences.

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