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Original Article

Effect of training with curcumin on liver Nrf2/HO-1 of rats exposed to cadmium

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Abstract

Background: Cadmium, a toxic heavy metal, has been shown to induce oxidative stress, leading to severe liver injury. The aim of the present study was to investigate the effect of aerobic exercise combined with curcumin consumption on the liver Nrf2/HO-1 pathway in mice exposed to cadmium.

Methods: 40 male Wistar rats aged 8-10 weeks (n=8 in each group) were allocated into five groups: control (C), cadmium (Cd), cadmium+curcumin (Cd+Cu), cadmium+training (Cd+AT), and cadmium+curcumin+exercise (Cd+Cu+AT). The Cd groups received 5 mg/kg of cadmium daily via drinking water. The AT groups underwent running sessions for eight weeks, five times per week, with each session lasting 30-60 minutes at a speed of 15 m/min on a 15-degree incline. Additionally, the daily intake of curcumin was 160 µL/kg administered orally.

Results: There was a significant increase in Nrf2 and HO-1 gene expression in Cd+Cu (p=0.038 and p=0.047, respectively), Cd+AT (p=0.035 and p=0.039, respectively) and Cd+Cu+AT (p=0.0001) compared to Cd. This increase was also observed in Cd+Cu+AT compared to Cd+Cu (p=0.038 and P=0.041, respectively) and Cd+AT (p=0.042 and P=0.049, respectively).

Conclusion: Aerobic training combination with curcumin may possibly inhibit cadmium-induced liver damage through up-regulating the Nrf2/HO-1 pathway.

Keywords: Exercise, Curcumin, Oxidative Stress Cadmium

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Introduction

Cadmium (Cd) is a heavy metal known to induce significant oxidative stress and damage to liver tissues, leading to various pathologies, including liver fibrosis and necroinflammation (1). The activation of nuclear factor erythroid 2-related factor 2 (Nrf2) and heme oxygenase-1 (HO-1) has been identified as critical mechanisms by which cells defend against cadmium-induced injury (2). The effect of aerobic training on liver Nrf2 and HO-1 expression in rats exposed to cadmium is a significant area of research, particularly given the increasing concerns over environmental pollutants and their impact on health. The exploration of aerobic exercise as a potential mitigative intervention presents a promising avenue for improving liver health amidst such toxic exposures. Research has demonstrated that aerobic exercise enhances the expression of protective proteins, such as nuclear factor erythroid 2-related factor 2 (Nrf2) and heme oxygenase-1 (HO-1), which play crucial roles in the liver's antioxidant response and overall metabolic regulation (3). In controlled experimental studies, rats subjected to aerobic training exhibited significant increases in Nrf2 and HO-1 levels, which correlated with reduced markers of liver dysfunction, including lower levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (4). This suggests that aerobic training not only supports the liver's adaptive mechanisms in response to oxidative stress but also promotes overall liver functionality. The potential of aerobic exercise as a non-pharmacological strategy to counteract the adverse effects of cadmium exposure highlights its relevance in the context of public health, especially for populations at risk due to environmental toxins (2). In addition, research has shown that curcumin can enhance the expression of Nrf2, a transcription factor that regulates the expression of various antioxidant proteins, including HO-1, thereby improving the liver's antioxidant capacity and protecting against oxidative damage (5). Notably, studies indicate that curcumin's administration leads to a significant reduction in liver damage markers and inflammatory cytokines, suggesting a multifaceted mechanism that includes both antioxidant and anti-inflammatory effects (6). This positions curcumin as a promising candidate for therapeutic intervention in cases of heavy metal toxicity and associated hepatic dysfunction. Controversies exist regarding the optimal dosage and administration routes of curcumin, as variations in these parameters can influence its efficacy in protecting liver health under toxic conditions (5). Moreover, the potential variability in response to curcumin treatment across different studies emphasizes the need for further investigation to establish standardized

protocols for its use. Understanding the interplay between exercise, curcumin, Nrf2, and HO-1 is vital for developing effective strategies to combat cadmium-induced liver damage and improve overall liver health in affected populations. In this study, an attempt was made to investigate the simultaneous effect of aerobic exercise and curcumin consumption on the Nrf2/HO-1 pathway of liver tissue in mice exposed to cadmium.

Material and methods

Animals

In this experimental study, 40 male Wistar rats, aged 8-10 weeks and weighing approximately 190-220 grams, were sourced from the laboratory animal breeding and reproduction centre. To facilitate adaptation to their new environment, the rats were housed in the laboratory for one week. They were maintained under standard conditions, including a 12:12 hour light-dark cycle, a relative humidity of 55%, a temperature range of 22 to 24°C, with unrestricted access to water and food. Following this acclimatization period, the rats were randomly allocated into eight groups: control (C), cadmium (Cd), cadmium+curcumin (Cd+Cu), cadmium+exercise (Cd+AT), and cadmium+curcumin+exercise (Cd+Cu+AT). This research received approval from the ethics committee at the Islamic Azad University, Ayatollah Amoli branch, under the code IR.IAU.AMOL.REC.1403.118.

Cadmium supplementation

Pure Cd chloride was sourced from Sigma Aldrich. Based on the number and weight of the rats, 35 mg of Cd was dissolved in the daily water intake of the groups receiving Cd. The rats were administered 5 mg/kg of Cd dissolved in their drinking water daily (7).

Training protocol

The rats were familiarized with the treadmill for a week, running for 10 minutes each day at a speed of 8 m/min; 0% slope. Subsequently, they ran daily for 60 minutes at a speed of 15 m/min on a 15-degree slope for eight weeks, with five sessions each week. To adhere to the principle of overload, the exercise duration was set at 30 minutes in the first to fourth week, then increased to 60 minutes from the fourth to the eighth week (8).

Curcumin supplementation

Initially, curcumin was sourced from Sigma Aldrich in America. Subsequently, each mouse was administered a solution of 160 µl of curcumin (dissolved in dextrose) per kilogram of body weight using small bottles (9).

Dissection and sampling

Forty-eight hours after the end of the protocol, animals were anesthetized after a 12-hour fast. Once full anesthesia was achieved, the liver tissue was dissected, weighed, and rinsed. The tissue after being frozen with liquid nitrogen, it was stored in a refrigerator at -80°C. To mitigate the influence of circadian rhythms, the tissue collection commenced at 8:00 AM and concluded at 11:30 AM. The total RNA was extracted from liver using RNA purification kits (Cinagene, Iran). Complementary DNA (cDNA) was measured based on the standard manufacturer's protocol.

Data analysis

One-way analysis of variance and Tukey's post hoc test was performed to evaluate the differences between the groups in SPSS software ($p<0.05$).

Results

Data analysis showed that there was a difference in the level of Nrf2 changes in liver between different groups ($p=0.0001$, $F=11.129$). The Tukey's test showed a decrease in the amount of Nrf2 in Cd compared to C ($p=0.0001$). Also, a significant increase was observed in Cd+Cu ($p=0.038$), Cd+AT ($p=0.035$) and Cd+Cu+AT ($p=0.0001$) compared to Cd; and Cd+Cu+AT compared to Cd+Cu ($p=0.038$) and Cd+AT ($p=0.042$) (Figure 1).

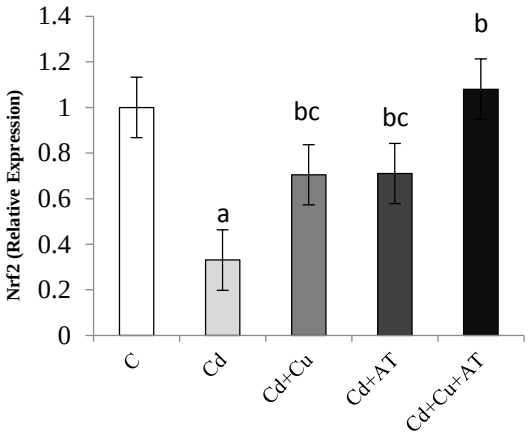

Figure 1. Liver expression of Nrf2 by one-way ANOVA (p<0.05).

a Difference from the C group, b Difference from the Cd group, c Difference from the Cd+Cu+AT group. C: control, Cd: cadmium, Cd+Cu: cadmium+curcumin, Cd+AT: cadmium+training and Cd+Cu+AT: cadmium+curcumin+training.

Also, data analysis showed that there is a significant difference in HO-1 expression of liver between different groups (p=0.0001, F=10.435). The Tukey's test results showed a significant decrease in HO-1 in Cd compared to C (p=0.0001). Also, a significant increase was observed in Cd+Cu (p=0.047), Cd+AT (p=0.039) and Cd+Cu+AT (p=0.0001) compared to Cd; and Cd+Cu+AT compared to the Cd+Cu (p=0.041) and Cd+AT (p=0.049) (Figure 2).

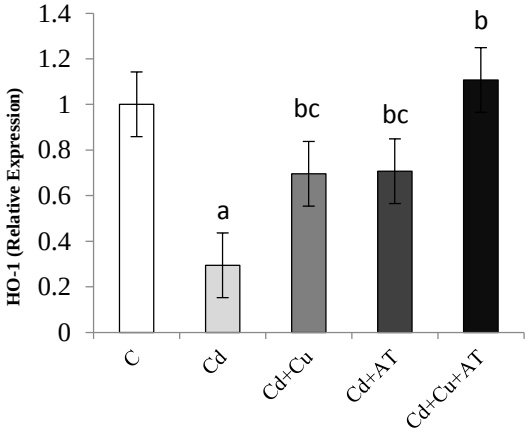

Figure 2. Liver expression of HO-1 by one-way ANOVA (p<0.05).

a Difference from the C group, b Difference from the Cd group, c Difference from the Cd+Cu+AT group. C: control, Cd: cadmium, Cd+Cu: cadmium+curcumin, Cd+AT: cadmium+training and Cd+Cu+AT: cadmium+curcumin+training.

Discussion

The results of the present study showed that cadmium exposure is associated with a decrease in antioxidant capacity. Cadmium exposure is associated with the generation of reactive oxygen species (ROS), such as hydroxyl radicals (HO·), superoxide radicals (O_2·), and hydrogen peroxide (H_2O_2) (10). These ROS overwhelm the cell's capacity to maintain a reduced state, resulting in oxidative stress that activates various signaling pathways, including the nuclear factor (erythroid-derived 2)-like 2 (Nrf2) pathway (10). The Nrf2/Keap1 pathway plays a critical role in cellular defense against oxidative stress by upregulating the expression of antioxidant genes, thus protecting cells from cadmium-induced damage (10). The cellular response to cadmium involves both activation of pro-inflammatory cytokines and a reduction in the activity of antioxidant enzymes (1, 10). Inflammatory responses are mediated by the activation of Kupffer cells and neutrophil infiltration, further exacerbating tissue injury through the release of additional pro-inflammatory mediators (10). The cumulative effect of these stressors can lead to conditions such as non-alcoholic fatty liver disease (NAFLD) and hepatic necroinflammation (10).

Aerobic exercise has been shown to enhance various physiological functions, particularly in the context of liver health and metabolic regulation. It plays a critical role in improving cardiovascular efficiency and overall oxygen delivery to body organs, including the liver, which is essential for maintaining metabolic homeostasis and preventing liver-related diseases (11, 12). Aerobic exercise also contributes to the modulation of the antioxidant system. It helps mitigate the oxidative stress induced by HFD through enhancing the activity of antioxidant enzymes like glutathione peroxidase (GSH-Px) (3). This is particularly relevant since obesity and high-fat diets are associated with increased production of reactive oxygen species (ROS) that can damage cellular structures, including mitochondrial function (3, 12). Our findings demonstrated that aerobic exercise significantly modulated the expression levels of both Nrf2 and HO-1 in the liver tissues of cadmium-exposed rats. Specifically, aerobic training resulted in a notable increase in Nrf2 protein levels, which suggests enhanced antioxidant response mechanisms. Furthermore, the elevation of HO-1 expression observed in these rats indicates a protective adaptation to oxidative stress induced by cadmium exposure (3). Cadmium exposure typically results in elevated levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), but aerobic training was effective in lowering these enzyme levels to near-normal ranges

(12). This reduction implies that exercise may ameliorate cadmium-induced hepatotoxicity by promoting liver health and functionality. The research suggests that aerobic exercise may serve as a non-pharmacological intervention to counteract the toxic effects of cadmium by enhancing the liver's adaptive response through the regulation of key protective factors such as Nrf2 (Nuclear factor erythroid 2-related factor 2) and HO-1 (Heme oxygenase-1). Previous studies have shown that aerobic training can modulate apoptotic pathways in the liver, particularly by influencing the balance between pro-apoptotic factors (like Bax) and anti-apoptotic factors (like Bcl-2) (2). In the context of cadmium exposure, the observed increase in Bcl-2 expression following endurance training indicates a potential protective effect against cadmium-induced apoptosis (2). This aligns with findings that suggest regular aerobic activity helps improve organ function by reducing oxidative stress and inflammation, both of which are exacerbated by cadmium toxicity (12). Additionally, the findings emphasize that moderate-intensity exercise can initiate beneficial adaptations in liver function over time. Engaging in activities such as walking, swimming, or cycling for a sustained duration has been associated with improved metabolic profiles, particularly for individuals with sedentary lifestyles (13).

The effects of curcumin on liver Nrf2 and HO-1 expression in rats exposed to cadmium have garnered significant attention in recent research. Cadmium exposure is known to induce oxidative stress and may lead to various hepatic pathologies. The inclusion of curcumin, a bioactive compound with antioxidant properties, has been proposed as a potential mitigator of cadmium-induced liver damage. The results of the present study showed that curcumin consumption was associated with increased Nrf2/HO-1 expression in the liver tissue of mice exposed to cadmium. Curcumin, a bioactive compound derived from turmeric, has been identified as a potent activator of the Nrf2 pathway, which plays a critical role in the antioxidant stress response and neuroprotection. Nrf2, upon activation, translocates into the nucleus and binds to the antioxidant response element (ARE) in DNA, leading to the transcriptional upregulation of various protective genes, including heme oxygenase-1 (HO-1) and superoxide dismutase (SOD) (10, 14). This pathway is crucial in mitigating oxidative stress, particularly in conditions such as liver damage induced by heavy metals like cadmium. Research has shown that curcumin can effectively activate the Nrf2/HO-1 signaling pathway, thereby enhancing the brain's antioxidant capacity and promoting recovery from oxidative stress and inflammation after brain injuries (14). In experimental

models involving blast brain injury, administration of curcumin resulted in significantly elevated levels of Nrf2 and HO-1 expression in the brain tissue, contrasting with lower levels observed in untreated injury groups. This indicates that curcumin not only activates Nrf2 but also contributes to the protective mechanisms afforded by HO-1, which is known for its antioxidant properties (6, 14). Moreover, curcumin's neuroprotective effects are thought to be mediated through its ability to modulate various signaling pathways. It has been observed to downregulate the pro-inflammatory transcription factor NF-κB, thus further supporting the anti-inflammatory effects associated with Nrf2 activation (14). The interplay between Nrf2 and NF-κB is significant, as Nrf2 can mitigate inflammatory responses while promoting the expression of antioxidant proteins that safeguard against cellular damage caused by reactive oxygen species (ROS) (14). The precise mechanisms by which curcumin exerts its beneficial effects through the Nrf2/HO-1 pathway remain an area of active investigation. However, the existing evidence strongly supports the notion that curcumin's ability to enhance Nrf2 and HO-1 expression is a vital component of its protective action against oxidative stress and inflammation, particularly in the context of liver damage due to cadmium exposure (6, 10, 14). Future studies are needed to further delineate the specific molecular interactions and pathways involved in curcumin's protective effects on hepatic tissues.

In the present study, the simultaneous effect of aerobic exercise and curcumin on Nrf2/HO-1 expression was greater than the effect of either alone. No studies have been observed that have examined the simultaneous effect of curcumin and aerobic exercise on these variables. However, Seddighi et al. (2020) showed that aerobic exercise combined with berberine chloride has an additive effect on improving oxidative stress markers in the heart tissue of diabetic rats with STZ (15). Also, Sahrai et al. (2020) observed that berberine chloride combined with aerobic exercise can inhibit STZ-induced liver damage, possibly through upregulation of the Nrf2/HO-1 and PPARγ pathways (16). It seems that aerobic exercise and curcumin, with their synergistic effects, have improved the expression of Nrf2/HO-1 in liver tissue.

Conclusion

The results of the present study showed that aerobic exercise and curcumin are a suitable therapeutic method to protect liver tissue against cadmium-induced damage. However, the

effect of combining aerobic exercise and curcumin had a better effect on the Nrf2/HO-1 pathway than either alone. It seems that aerobic exercise and curcumin, by increasing Nrf2 gene expression, upregulate HO-1 expression and can improve liver function in mice exposed to cadmium. Therefore, it is recommended to pay more attention to the simultaneous effect of exercise as a non-pharmacological treatment method and the use of natural antioxidant and anti-inflammatory supplements such as curcumin in the control of cadmium-induced liver damage.

Declarations

Ethical Considerations

Compliance with ethical guidelines

This research was carried out with the approval of the Ethics Committee of the Research Institute of Physical Education and Sports Sciences with code IR.IAU.AMOL.REC. 1403.118.

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Authors' contributions

Conceptualization: Ahmad Abdi, Mina Fallah; Methodology: Ahmad Abdi, Mina Fallah; Formal analysis: Ahmad Abdi; Investigation; Writing: Ahmad Abdi; Funding acquisition: Mina Fallah.

Conflicts of interest

The authors declare that they have no competing interests.

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