Research Paper: The Effect of Resistance Training and Berberine Chloride on the Apoptosis-related Unfolded Protein Response Signaling Pathway in the Hippocampus of Diazinon-poisoned Rats

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Introduction: Diazinon is one of the most widely-used organophosphate pesticides in the world. This toxin enters the body in various ways and induces oxidative stress in various tissues. It has been proved that activation of Unfolded Protein Response (UPR) under oxidative stress is a steady mechanism for maintaining cell function and survival. Therefore, the present study aimed to review the effect of Resistance Training (RT) and Berberine Chloride (BC) on the apoptosis-related UPR signaling pathway in the hippocampus of diazinon-poisoned rats.

Methods: In this experimental study, 40 male Wistar rats weighing 250 ±50 g were randomly divided into eight groups of five rats of 1) diazinon + 2 mg/kg BC + RT, 2) diazinon + 15 mg/kg BC + RT, 3) diazinon, 4) diazinon + RT, 5) diazinon + 2 mg/kg BC, 6) diazinon + 15 mg/kg BC, 7) healthy control, and 8) sham. The groups were treated for 5 weeks. At the end of the fifth week, \textit{ATF-4}, \textit{ATF-6}, and \textit{CHOP} gene expression in hippocampus tissue were measured by quantitative real-time RT-PCR.

Results: Diazinon significantly increased the expression of \textit{ATF-4}, \textit{ATF-6}, and \textit{CHOP} gene expression in hippocampus tissue were measured by quantitative real-time RT-PCR.

Conclusion: This study showed that RT and BC have a protective effect against diazinon-induced toxicity in the hippocampus.

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1. Introduction

In the last five decades, pesticides have become an integral part of agriculture in the world (Jeyaratnam, 1990). Organophosphate (OP) compounds are commonly used as pesticides in all communities due to their affordability, availability, and rapid effect immediately after use (Leibson & Lifshitz, 2008). After using this product in crops and plants, diazinon is readily washed from surface waters, enters the groundwater, and eventually the aquatic environment (Dutta & Meijer, 2003). Because of its release into the aquatic environment, diazinon affects many organisms such as invertebrates, mammals, birds, and especially aquatic species such as fish (Hamm & Hinton, 2000). In recent years, research on the cholinergic effects of OPs in the brain has been more clinical. Particular attention has been paid to the involvement of cholinergic effects of OPs in the brain and the induction of oxidative stress and neurotoxicity. Neurotransmission loss is a major factor in the pathology of secondary nerve injury, as well as the long-term psychological and neurological consequences after OP exposure (Chen, 2012). The mechanism of action that underlies OP poisoning is the induction of oxidative stress and the inhibition of Acetylcholinesterase (AChE) activity, which may lead to the accumulation of acetylcholine in synaptic junctions, cholinergic syndrome, and eventual death. Thus, diazinon exposure has a chronic toxic effect on the Central Nervous System (CNS) and reduces the number of neurons (Hsieh, Deng, Ger & Tsai, 2001).

Recent findings have shown that AChE plays a role in inducing apoptosis (Aluigi, Guida & Falugi, 2010), but its mechanism is still unknown. It has already been shown that OP compounds cause oxidative stress and thereby inducing Reactive Oxygen Species (ROS), which can induce apoptosis in mammalian species. Diazinon has been shown to increase bax/bcl2 and caspase-3 ratios in heart tissue (Razavi, Hosseinzadeh, Movassaghi, Imenshahidi & Abnous, 2013). The Endoplasmic Reticulum (ER) is a multifunctional intracellular organelle and a key site required for protein synthesis, folding, transport of proteins, calcium homeostasis, and lipid biosynthesis (Wang et al., 2018). Any abnormality in the ER harms the process of protein biosynthesis and its modification and resulting in the production of incorrect proteins. Most incorrect proteins have no specific function in cellular metabolism. Thus, they are subjected to deformation or degradation (Cao, 2015). ER is one of the most important and multifunctional organelles for cell survival (Kara & Oztas, 2019), and ER stress is caused by the accumulation of unfolded proteins in the ER. This stress can reduce the production of functional proteins and even apoptosis. To save the cell from ER stress, an evolutionarily protected mechanism called Unfolded Protein Response (UPR) is activated (Grootjans, Kaser, Kaufman & Blumberg, 2016).

The molecular components of the UPR signaling pathway have been well studied over the past few decades. It is now known that in response to ER stress, three signal transducers present in the ER membrane are activated to initiate adaptive responses. These transducers include both protein kinase Inositol-Requireing kinase 1 (IRE1), double-stranded RNA-activated protein kinase-like ER

Highlights

- Diazinon significantly increased the expression of Activating Transcription Factor 4 (ATF-4), Activating Transcription Factor 6 (ATF-6), and CHOP in the hippocampus tissue of rats.
- Supplementation of berberine chloride with resistance exercise training significantly decreased ATF-4, ATF-6, and CHOP in the hippocampus tissue of rats exposed diazinon.

Plain Language Summary

Diazinon is one of the most widely used organophosphate pesticides in the world. This toxin enters the body via various ways and induces oxidative stress in different tissues. It has been proved that activation of Unfolded Protein Response (UPR) under oxidative stress is a reliable mechanism for maintaining cell function and survival. This study examined the effects of berberine chloride with resistance exercise on the apoptosis-related UPR signaling pathway in the hippocampus of diazinon-poisoned rats. The findings of this study showed that diazinon significantly increased the expression of ATF-4, ATF-6, and CHOP in the hippocampus tissue of rats. About 15 mg/kg berberine chloride with resistance training significantly decreased these gens, which may indicate a decrease in the rate of apoptosis in the hippocampus.
Kinase (PERK) and Activating Transcription Factor 6 (ATF6) transcription factor (Harding, Zhang, Bertolotti, Zeng & Ron, 2000; Yoshida, Matsu, Yamamoto, Okada & Mori, 2001). By suppressing general translation, the UPR from these three pathways produces the production of chaperones to correct the folding of ER aggregation proteins, reset Endoplasmic Reticulum-Associated protein Degradation (ERAD), reduces ER stress, and repair of ER function. However, if the stress exceeds the ER folding capacity, specific apoptotic programs (pro-apoptotic UPR) are activated, leading to cell death and apoptosis (Rutkowski & Hegde, 2010). In response to ER stress, PERK and IRE1α are activated via auto-phosphorylation. Activated PERK activates eukaryotic Initiation Factor 2 (eIF2α), which can selectively induce expression of ATF4 and CHOP pro-apoptotic transcription factors (Fribley, Zhang & Kaufman, 2009).

It has also been reported that activation of UPR subject to oxidative stress is a reliable mechanism for maintaining cell function and survival. Ongoing oxidative stress initiates apoptotic cascades and plays an important role in the pathogenesis of various human diseases, including diabetes, atherosclerosis, and neuronal diseases (Malhotra & Kaufman, 2007). Berberine Chloride (BC), an organic yellow and bitter-tasting alkaloid, has been used in traditional Chinese medicine for nearly 3000 years (Cai, Wang & Yang, 2016). Regarding multiple effects of BC (some of them enhance the factors of nerve protection pathways and others neutralize those that increase nerve damage), many unknown effects require a lot of research (Jiang, Li & Li, 2015). BC administration for nerve damage, many unknown effects require a lot of research (Jiang, Li & Li, 2015). BC administration for treating multiple human body disorders has been studied because it has antioxidant, cholinergic, antidiabetic, and anticancer properties (Gulfraz et al., 2008; Kulkarni & Dhir, 2010; Zhou & Mineshita, 2000). The ability of BC to cross the blood-brain barrier and its antioxidant potential has led to the use of BC in various neurological diseases. Also, BC can inhibit the process of oxidative stress in the central nervous system (Wang et al., 2004). Therefore, we hypothesized that BC might protect the brain, especially the hippocampus, against diazinon-induced toxicity. It has been shown that different exercises have various and specific physiological effects. Resistance Training (RT) is one of the most common training methods used by all training groups. It has been proven that the beneficial effects of RT on the brain can enhance the executive function of long-term and short-term memory (Cassilhas et al., 2007).

Regular training can reduce oxidative stress and reverse mitochondrial function and ER stress. Training-induced metabolic stress can activate the UPR because muscle contraction is directly involved in its activation. In other words, regular and moderate intensity training acts as a protective mechanism against stressors (Estébanex, de Paz, Cuevas & González-Gallego, 2018). In the present study, rats were exposed to diazinon compounds to induce neurotoxicity in the CNS, and then the combined effects of RT and BC on the hippocampus of the rats were investigated. Since the diazinon has a significant effect on the induction of apoptosis in neurons, its use is valuable for studying cell death in the CNS. To evaluate the efficacy of BC and RT, the changes of ATF-4, ATF-6, and CHOP/GADD153 gene expression were investigated as influencing factors on apoptosis in UPR signaling pathway in diazinon-induced toxicity in rats.

2. Methods

2.1. Study animals

In this experimental study, 40 male Wistar rats (40-50 weeks old) with a Mean±SD weight of 250±50 g were purchased and transferred to the Animal Research Center of Islamic Azad University, Central Tehran Branch, Tehran, Iran. All animals were housed in standard laboratory conditions in cages measuring 15 × 15 × 15 cm made of transparent polycarbonate (4 rats per cage) in controlled conditions (ambient temperature 22±2°C, 50±5% humidity, and 12/12 light/dark cycle) and free access to adequate water and food. All experiments were performed in accordance with the laboratory guidelines for the care and use of animals in Iran as well as the guidelines of the Animal Care Ethics Committee of the Research Institute of Sport Sciences (Ethical code: IR.SSRI.REC.1396.159).

The study rats were randomly divided into 8 groups, including G1: diazinon + BC (2 mg/kg) + RT, G2: diazinon + BC (15 mg/kg) + RT, G3: diazinon, G4: diazinon + RT, G5: diazinon + BC (2 mg/kg), G6: diazinon + BC (15 mg/kg), G7: control, and G8: normal saline (sham).

2.2. Diazinon induction

Liquid diazinon poison was purchased from Sigma USA. Diazinon was injected into rats at a dose of 1.5 mg/kg body weight intraperitoneally. Latex gloves and filter masks were used from the beginning to the end of the injection. Dilution was performed with normal saline 0.09. To prevent the toxin from spreading in ambient air during the preparation time, a laminar laboratory hood and insulin syringe (for each rat) were used.
2.3. Berberine Chloride (BC)

Berberine Chloride (BC) was purchased from Sigma USA in powder form. A digital weighing scale with 0.001 g precision was used to weigh BC. The doses of 2 and 15 mg/kg body weight BC were administered intraperitoneally for 5 weeks (5 days per week). BC was diluted in normal saline 0.09 (50 mg of BC in 1 mL of normal saline). The solution of BC was prepared using a magnet on a styrene machine without heat. Insulin syringes were used for the injection of BC.

2.4. Training protocol

RT was performed one session per day, 3 days per week for 5 weeks. It consists of climbing up a wooden vertical ladder with 26 steps (100 cm in height between 2 cm steps and an 80-degree slope) and carrying weights attached to rats’ tails. To familiarize the rats with the ladder, they climbed the ladder one week before starting the training program without carrying any weights. Each training session consisted of 2 sets of 6 repetitions that required 8-12 active moves per climb. The rest was 60 seconds between each repetition and 2-3 minutes between each turn. Weights started at 10% of total body weight in the first week, 20% in the second week, 30% in the third week, 40% in the fourth week, and 50% in the fifth week. The weights were measured at the beginning of each training week, and the new weights that rats were supposed to carry were adjusted to their weight each week. The training protocol is presented in Table 1.

2.5. Sampling and hippocampus tissue extraction

For tissue extraction, at the end of 5 weeks of RT and 48 hours after the last training session, the rats were become unconscious with an intraperitoneal injection of a combination of ketamine (75 mg/kg) (Sigma, US) and xylene (10 mg/kg) (Sigma, US). For collecting the hippocampus tissues, the heads of rats were separated from the neck by special scissors. At first, using a razor, the skull was cleaved, and the brain was carefully removed. After being placed in cold saline solution, cooling, and tissue fixation, the brain was divided precisely from the midline to half, and the hippocampus was separated from the limbic system based on hippocampal coordinates using the Atlas of Clean Sinus. Hippocampus tissues were quickly placed on a cryotube and transferred to 80°C, and they were kept at the same temperature until testing. Finally, the quantitative real-time RT-PCR was used to measure the research variables.

2.6. Gene expression measurement using quantitative real-time RT-PCR

Five rats in each group were used to study the expression of target genes. Total RNA was extracted from hippocampus tissue by TRIzol solution (KIAAZOL, USA) in a clean RNase-free microtube, and a NanoDrop device was used to measure RNA concentration. The cDNA was synthesized using a specific kit (BioFact) according to its instructions. First, 500 ng of RNA was mixed with 10 µL of the kit (including reaction buffer, dNTP, random hexamer and oligo dT primers, nuclease inhibitor, and reverse transcriptase enzyme) and finalized with water without nucleic enzymes. Place the mixture for 40 minutes at 42°C and then for 5 minutes at 85°C. To determine the expression levels of the ATF-4, ATF-6, and CHOP/GADD153 genes (its gene called Ddit3), the specific primers were designed by Primer3 software and verified for functional specificity in the NCBI BLAST tool. The primers used in this study are shown in Table 2. GAPDH was used as the reference gene in each sample for normalization. The BioFact kit was used for real-time RT-PCR according to the kit protocol. The reaction was performed by Applied Biosystem StepOne. A thermal program for the reaction was performed for 15 min at 95°C, 20 s at 95°C, 1 min at 60°C for 40 cycles. ΔΔCT-2 (fold change) method was used to determine the relative expression of the target genes. The Cycle Threshold (CT) of the samples was compared with internal control CT (GAPDH). In this method, the ΔCT value is obtained from the difference between the CT genes and the CT genes of the GAPDH control. ΔΔCT was calculated after subtracting Δ-CT (target group) from Δ-CT values (control group), and then 2^{-ΔΔCT} was calculated.

2.7. Statistical analysis

All data were reported using mean and standard deviation. One-way ANOVA was used to examine the changes between the healthy and patient groups that received no medication. For treated patient groups, 2-way ANOVA was used. Also, the Tukey post hoc tests were used to compare the differences between the two groups. The obtained data were analyzed using GraphPad Prism 8 software. P-values less than 0.05 were considered statistically significant.

3. Results

The first finding of this study showed that the ATF-4 gene expression levels significantly increased by di-azinon poisoning (P<0.001). There was no significant difference in expression of this gene between healthy control and sham groups (P>0.99) (Figure 1A). RT significantly decreased ATF-4 gene expression compared to the patient group that received no treatment (P<0.01).
BC also reduced the expression of this gene in a dose-dependent manner at concentrations of 2 mg/kg (P<0.001) and 15 mg/kg (P<0.001). RT and BC could enhance the effect of each other on the expression of this gene and significantly reduced its expression (P<0.001) (Figure 2A).

Comparing the mean expression of the ATF-6 gene in the patient group with the healthy control and sham groups showed a significant increase in the expression of this gene in the patient group compared to the healthy and sham groups (P<0.001). But there was no significant difference between healthy control and sham groups (P>0.99) (Figure 1B).

Figure 1. Influences of diazinon poison
A: ATF-4; B: ATF-6; and C: CHOP genes compared to the control and sham groups in hippocampus tissue.
Data are presented as mean and standard deviation; ***P<0.001.

The comparison between the mean scores of ATF-6 gene expression in the disease group that received no treatment, RT (P<0.05), BC 2 mg/kg (P<0.01), and BC 15 mg/kg (P<0.001) decreased the gene expression independently. Also, the interaction of BC 2 mg/kg (P<0.001) and BC 15 mg/kg (P<0.001) and RT with a greater effect reduced this decrease (Figure 2B).

Comparison of CHOP gene expression in the patient, healthy control, and sham groups showed an increase in the patient group as in other genes (P<0.001), but in the sham and healthy control groups, this expression was very low, and there was no significant difference between them (P>0.99) (Figure 1C). For the CHOP gene,

Table 1. Resistance training protocol

<table>
<thead>
<tr>
<th>Training Duration</th>
<th>Number of Sessions Per Week</th>
<th>Movement</th>
<th>Sets and Repetitions in Each Session</th>
<th>Number of Movements in Each Repetition</th>
<th>Rest Between Each Repetition</th>
<th>Rest Between Each Set</th>
<th>% Body Weight</th>
</tr>
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<tr>
<td>5</td>
<td>3</td>
<td>Climbing the latter</td>
<td>2 × 6</td>
<td>8-12</td>
<td>60 s</td>
<td>2-3 min</td>
<td>10-50</td>
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</table>
Table 2. Specific primers used in the real-time RT-PCR step

<table>
<thead>
<tr>
<th>Name</th>
<th>Primer</th>
<th>Gene Bank Number</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATF-4</td>
<td>F: 5′-TCAGACACAGGCAAGGGAGGA-3’</td>
<td>NM_024403.2</td>
<td>287</td>
</tr>
<tr>
<td></td>
<td>R: 5′-GAACAGGGGAAGGCTGCAAGA-3’</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATF-6</td>
<td>F: 5′-AGGTGCTGGAGTTAAGATGGAG-3’</td>
<td>NM_001107196.1</td>
<td>216</td>
</tr>
<tr>
<td></td>
<td>R: 5′-AGTGACAGGAAAGACAGGAG-3’</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHOP/Dec3</td>
<td>F: 5′-AGCTGGAAGCATGGTATGAGGA-3’</td>
<td>NM_001109986.1</td>
<td>132</td>
</tr>
<tr>
<td></td>
<td>R: 5′-CAAGGGATGCAGGGTCAAGGAG-3’</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GAPDH</td>
<td>F: 5′-AAGTTGAGGCAGCCAGCCCAAGG-3’</td>
<td>NM_017008</td>
<td>121</td>
</tr>
<tr>
<td></td>
<td>R: 5′-CATACTGCACACCGATCCGACC-3’</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 2. Influences of diazinon poison

A: Changes of ATF-4; B: ATF-6; and C: CHOP genes in the hippocampus tissue in research groups.

Data are presented as mean and standard deviation. Compared to diazinon + RT group; *P<0.05, **P<0.01, ***P<0.001.

Compared to diazinon without RT, #P<0.05, ##P<0.01, ###P<0.001.
Basic and Clinical Neuroscience

This study shows that RT can decrease the expression of the OXPHOS system (Hong, Kim, Kim & Park, 2017). This time sequence is likely to have implications for ER inactivation of the IRE1α, ATF6, and PERK pathways. Long ER stress results in sequential activation and then event, the activation time can vary. Specifically, pro-apoptotic genes involved in the UPR signaling pathway due to diazinon poisoning in the hippocampus tissue of rats, which is consistent with many studies. Diazinon, as an organophosphorus toxin, causes oxidative stress in various tissues by over-producing ROS and reducing antioxidant factors in cells (Lukaszewicz-Hussain, 2010). Since diazinon increases ROS, numerous studies have shown that exposure to OPs, such as diazinon, can lead to neural cell death by introducing apoptotic pathways (Voorhees, Rohman, Lein & Pieper, 2017). The evidence suggests that protein folding and ROS production are related events. However, this domain of ER stress has not been well explored. Since oxidative protein folding occurs in the ER and disruption of protein folding can have adverse consequences, ROS production can directly or indirectly affect ER homeostasis and protein folding (Malhotra & Kaufman, 2007). The ability of cells to respond to perturbations in ER function or “ER stress” is crucial for cell survival, but chronic or untreated ER stress can lead to apoptosis (Tabas & Ron, 2011). ER stress is sensed by the three upstream proteins of IRE1α, ATF6, and PERK that initiate a cascade of remedial actions upon activation. The activity of these three pathways generally constitutes the UPR (Ron & Walter, 2007). Although all three pathways are usually activated by any ER stress event, the activation time can vary. Specifically, prolonged ER stress results in sequential activation and then inactivation of the IRE1α, ATF6, and PERK pathways. This time sequence is likely to have implications for ER stress-induced apoptosis (Lin et al., 2007). On the other hand, ATF4 activation, induced by PERK phosphorylation, plays an essential role in inducing CHOP in response to ER stress. Overexpression of CHOP increases apoptosis (Nishitoh, 2012).

Many studies report that exercises can effectively improve various types of ER stress-related injuries such as obesity, diabetes, neurodegenerative diseases, hypoxia, and sarcopenia in skeletal muscle, liver, brain, and cardiovascular systems (Hong, Kim, Kim & Park, 2017). This study shows that RT can decrease the expression of apoptosis-related genes in the UPR signaling pathway that was enhanced by the increase in free radicals induced by diazinon toxin, which is consistent with many studies. Twelve weeks of aerobic training and RT reduced ER stress in obesity by weakening the GRP78 signaling network (Khadir et al., 2016). High-intensity 5-week physical training increased mitochondrial biogenesis and decreased ER stress, as well as decreased expression of CHOP in the apoptotic pathway in rat skeletal muscle tissue (Kim et al., 2014). Also, an 8-week treadmill reduced CHOP levels in the hippocampus of stress-resistant mice (Kang, 2015). Treadmill exercise improved cardiac function and reduced myocardial infarction by decreasing the expression of GRP78, DER-LIN-1, p-PERK, p-eIF2α, ATF4/6, XBP1, CHOP, and caspase-3 (Bourdier et al., 2016).

Previous studies have shown that BC suppresses the pro-apoptotic signal by inhibiting caspase-3 and NF-kB as well as stimulating Bcl-2 expression (Chai et al., 2014). Also, when examining the neuroprotective effect of BC on mercury chloride-induced oxidative stress and neurotoxicity in mice, the results showed that BC improved antioxidant protection and had anti-apoptotic and photo-protective properties (Abdel Moneim, 2015). Another study provides in vitro strong evidence that BC can prevent oxidative stress due to hypoxia or oxidation. The mechanisms involved can be attributed to the inhibition of both the ER and mitochondrial-dependent pathways (Yu et al., 2013). The present study results also showed that BC, with its antioxidant effects, can decrease diazinon-induced oxidative stress by decreasing the expression of genes involved in apoptosis, including ATF-4, ATF-6, and CHOP.

In line with the results of previous studies, the results of this study also show the positive and synergistic effects of RT and BC in reducing the expression of ER stress-related genes due to diazinon toxin oxidative stress. In this study, the expression of ATF-4, ATF-6, and CHOP genes at the mRNA level was investigated. Since the function of these genes is determined at the protein level, it is recommended to study the protein levels in respective groups.

The results showed that RT with BC inhibited the expression of genes involved in ER stress so that the expression levels of ATF-4, ATF-6, and CHOP genes had a dose-dependent decreasing trend. Based on the previous findings and the present study, the UPR signaling pathway can be an effective signaling pathway in apoptosis induced by organophosphorus toxins such as diazinon in the hippocampus. Therefore, it is recommended to use

4. Discussion

Overall, this study showed a significant increase in the expression of ATF-4, ATF-6, and CHOP genes in the UPR signaling pathway due to diazinon poisoning in the hippocampus tissue of rats, which is consistent with many studies. Diazinon, as an organophosphorus toxin, causes oxidative stress in various tissues by over-producing ROS and reducing antioxidant factors in cells (Lukaszewicz-Hussain, 2010). Since diazinon increases ROS, numerous studies have shown that exposure to OPs, such as diazinon, can lead to neuronal cell death by introducing apoptotic pathways (Voorhees, Rohman, Lein & Pieper, 2017). The evidence suggests that protein folding and ROS production are related events. However, this domain of ER stress has not been well explored. Since oxidative protein folding occurs in the ER and disruption of protein folding can have adverse consequences, ROS production can directly or indirectly affect ER homeostasis and protein folding (Malhotra & Kaufman, 2007). The ability of cells to respond to perturbations in ER function or “ER stress” is crucial for cell survival, but chronic or untreated ER stress can lead to apoptosis (Tabas & Ron, 2011). ER stress is sensed by the three upstream proteins of IRE1α, ATF6, and PERK that initiate a cascade of remedial actions upon activation. The activity of these three pathways generally constitutes the UPR (Ron & Walter, 2007). Although all three pathways are usually activated by any ER stress event, the activation time can vary. Specifically, prolonged ER stress results in sequential activation and then inactivation of the IRE1α, ATF6, and PERK pathways. This time sequence is likely to have implications for ER stress-induced apoptosis (Lin et al., 2007). On the other hand, ATF4 activation, induced by PERK phosphorylation, plays an essential role in inducing CHOP in response to ER stress. Overexpression of CHOP increases apoptosis (Nishitoh, 2012).

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RT with BC to protect the hippocampus from injuries induced by diazinon poisoning.

Ethical Considerations

Compliance with ethical guidelines

All experiments were performed by the laboratory guidelines for the care and use of animals in Iran and the Animal Care Ethics Committee guidelines of the Research Institute of Sport Sciences (Ethical code: IR.SSRI.REC.1396.159). Protocols and guidelines followed by the National Institutes of Health (NIH) to care for and use experimental animals.

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Authors’ contributions

Conceptualization and supervision: Mohammad-Ali Azarbajjan; Acquisition of animal data: Ali Esfandiarifar and Seyed Behnamedin Jameie Tale; Igor analysis: Igor Azarbayjani; Acquisition of animal data: Ali Esfandiarifar and Seyed Behnamedin Jameie Tale; Analysis and approving the final version for publication: All authors.

Conflict of interest

The authors declared no conflict of interest.

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