Chinese Journal of Integrative Medicine

Available online at link.springer.com/journal/11655
Journal homepage: www.cjim.cn/zxyjhen/zxyjhen/ch/index.aspx
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Original Article

Berberine Hydrochloride Improves Cognitive Function and Hippocampal Antioxidant Status in Subchronic and Chronic Lead Poisoning*

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ABSTRACT **Objective:** To determine the neuroprotective effects of berberine hydrochloride (BBR) against lead-induced injuries on the hippocampus of rats. **Methods:** Wistar rats were exposed orally to doses of 100 and 500 ppm lead acetate for 1 and 2 months to develop subchronic and chronic lead poisening models, respectively. For treatment, BBR (50 mg/kg daily) was injected intraperitoneally to rats poisoned with lead. At the end of the experiment, the spatial learning and memory of rats were assessed using the Morris water maze test. Hippocampal tissue changes were examined by hematoxylin and eosin staining. The activity of antioxidant enzymes catalase, superoxide dismutase, glutathione peroxidase, and malondialdehyde levels as parameters of oxidative stress and antioxidant status of the hippocampus were evaluated. **Results:** BBR reduced cognitive impairment in rats exposed to lead ($P<0.05$ or $P<0.01$). The resulting biochemical changes included a decrease in the activity of antioxidants and an increase in lipid peroxidation of the hippocampus of lead-exposed rats ($P<0.05$ or $P<0.01$), which were significantly modified by BBR ($P<0.05$). BBR also increased the density of healthy cells in the hippocampus of lead-exposed rats ($P<0.05$). Significant changes in tissue morphology and biochemical factors of the hippocampus were observed in rats that received lead for 2 months ($P<0.05$). Most of these changes were insignificant in rats that received lead for 1 month. **Conclusion:** BBR can improve oxidative tissue changes and hippocampal dysfunction in lead-exposed rats, which may be due to the strong antioxidant potential of BBR.

KEYWORDS lead acetate, berberine, hippocampus, oxidative stress, Morris water maze

Lead is a heavy metal that is abundant in the environment. This metal remains in the body and accumulates and exerts its toxic effects by binding to ligands necessary for physiological functions.[1] Lead has a long biological half-life due to its very low excretion rate. Almost all organs of the body are affected by lead. Disorders of the nervous system are common occurrences during lead poisoning.[1,2] Prolonged exposure to low doses of lead in humans, especially children, impairs cognitive function, learning and intelligence in children. High doses of lead can even cause severe nerve damage that can lead to death.[3] Lead, directly or specifically by activating inflammatory factors and oxidants, can cause irreversible damage to all cell components, including proteins, fats, and nucleic acids.[2,4] Activation of oxidative stress is one of the main harmful pathways during lead posioning.[4] Therefore, the use of substances that have the ability to remove oxidants or activate intracellular antioxidants can be useful in reducing lead damage.

Berberine hydrochloride (BBR) is a natural isoquinoline alkaloid isolated from plants such as *Coptis chinensis* (Huanglian) and *Hydrastis canadensis* (Baimaogen).[5] A broad spectrum of effective pharmacological activities of BBR in clinical and experimental studies have been investigated, including anti-inflammatory, antioxidant, anti-diabetic, analgesic, and anti-arrhythmic influences.[5-7] The neuroprotective effects of BBR in various disorders of the nervous system, especially cerebral ischemia, Alzheimer's disease, Parkinson's disease, and multiple sclerosis have been studied.[7-9] According to

*Supported by the Research Project of Shahid Sadoughi University of Medical Sciences and Health Services (No. 5894)

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DOI: https://doi.org/10.1007/s11655-024-3907-1

the above, in the present study, the effect of BBR on hippocampal structure and function in rats exposed to lead was investigated.

METHODS

Animals and Grouping

In this study, 72 male Wistar rats (200–220 g, 10 weeks old, specific-pathogen-free grade) which were purchased from the Department of Laboratory Animals of Pasteur Institute and were randomly divided into 9 groups using simple randomization: Group A received normal saline daily by oral gavage; Groups B and C were exposed to 100 and 500 ppm of lead acetate (CAS No. 215902, Sigma Aldrich, Germany) through drinking water, and Groups D and E were lead groups which were injected intraperitoneally (i.p.) with 50 mg/kg BBR (Cat. No. 141433-60-5, Merck, Germany) daily for 30 days. The remaining 4 groups were lead and BBR-treated groups that received lead and BBR for 2 months. At all stages of the experiment, the animals were maintained under standard light and dark conditions of 12 h/12 h, with suitable humidity (60%±10%) and temperature (22±2 ℃) and free access to water and food. All ethical principles were observed in accordance with the principles of working with laboratory animals approved at Shahid Sadoughi University of Medical Sciences in Yazd (ethical code: IR.SSU.MEDICINE.REC.1396.317).

Morris Water Maze Test

In the last week of the experiment, the Morris water maze (MWM) test was performed to assess spatial learning and memory in animals. The steps of this test were in accordance with the protocol implemented in previous studies.[10,11] In short, the water maze consists of 4 hypothetical quarters in which the animals were released into the water from 4 directions each day. In this way, 4 trials were performed every day. The time and distance traveled to reach the platform was observed by a video tracking camera and recorded in the Neuro-vision video tracking software (Tejhiz Gostar Omid Iranian Company, 2023 version, Iran). After 48 h of rest, to examine the spatial memory of the animals, the platform was removed from the maze and the time and distance traveled in the target quarter were recorded and compared in different groups.

Activity of Antioxidant Enzymes in Hippocampus

At the end of the trial period, 5 rats from each group were deeply anesthetized with chloral hydrate (350 mg/kg, i.p.). After decapitation, the animal's brains were immediately immersed in ice-cold phosphate-buffered saline (PBS) and the hippocampus was carefully and rapidly isolated from surrounding tissues. After homogenization, the tissue sample was centrifuged with 10% (w/v) ice-cold PBS and its supernatant was isolated for biochemical factors. All antioxidant enzymes [catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPX)] were assessed using Zellbio Company kits (Cat. No. ZB-CAT-96A, ZB-SOD-96A, ZB-GPX-96A; Germany). To measure CAT activity, homogenized tissue samples were added to microplate wells. The assay buffer and H_2O_2 reagent were then added and incubated for 1 min at 37 ℃. Finally, after adding chromogen and diluent solution, the optical density (OD) of the samples was read at 405 nm. To measure SOD, which is one of the most effective intracellular antioxidant enzymes and catalyzes the reaction of radical conversion of superoxide anion to oxygen and hydrogen peroxide, the homogenized tissue was used as the CAT method. Samples were added to wells and the test steps were performed according to the kit protocol that was also done in previous researches.[9,10] At the end, the light absorption of the samples was read at 0 and 2 min at 420 nm. GPX activity was also evaluated according to the kit protocol. For this purpose, after adding the samples to the wells, Reagent 2 solution was added to the samples and the samples were centrifuged at 4,000 r/min for 10 min. Then, Reagent 3 solution was added to the samples and incubated for 5 min at room temperature. Finally, the microplates were placed in enzyme-linked immunosorbent assay reader (Box998, Biotech Instrument Model, USA) and the OD was read at 412 nm.[9,10]

Malondialdehyde Level in Hippocampus

Malondialdehyde (MDA) is a substance resulting from lipid peroxidation. The basis of MDA measurement is the binding of thiobarbituric acid (TBA) to it and the formation of a chromogenic pigment. The Zellbio kit (Cat. No. ZB-MDA-96A) was also used to measure MDA. First, 8 standard solutions with a certain concentration were prepared. Then, 50 μL of the standard and separated samples were added to the vials and 50 μL of TBA solution was added to them. After that, 1 mL of chromogen solution was added. All vials were placed at boiling temperature for 1 h, then incubated for 30 min at 0 ℃ and centrifuged at 4,000 r/min for 10 min at 0 ℃. Then the light absorption of the samples was read at 535 nm. Finally, the

concentration of MDA was calculated based on the standard diagram.[9,10]

Histological Assessment of Hippocampus

After deep anesthesia, 3 animals from each group were perfused intracardically with 10% formalin. The animal's brain was completely removed and immersed in fixative solution for 48 h. For paraffinization, the samples were placed in a tissue processor for 24 h and finally the samples were molded. Consecutive coronal sections (4 sections 30 μm apart) were taken at the level of 2.7 mm of bregma. According to the previous protocol, the sections were stained using hematoxylin and eosin (HE) staining and pyramidal neurons in the CA1 subregion of the hippocampus with distinct nucleus and nucleoli were counted as healthy neurons under a 400× magnification light microscope (Olympus, USA).[10]

Statistical Analysis

All data were expressed as mean ± standard error ($\bar{x} \pm SE$) and were analyzed by GraphPad Prism software version 6. Data analysis of spatial learning was done with two-way analysis of variance and Ben Feroni's post-test. Analysis of other data was performed with one-way analysis of variance and Tukey *post hoc* test. $P<0.05$ was considered statistically significant.

RESULTS

Results of Spatial Learning and Memory by MWM Test

In the first 4 days, for the rats that exposed to lead for 1 month, only a dose of 500 ppm caused learning impairment in the form of an increase in the time and distance to reach the platform ($P<0.05$, Figures 1A and 1B). And learning impairment was also observed in rats that received lead of 500 ppm for 2 months ($P<0.05$ or $P<0.01$, Figures 1C and 1D). BBR improved learning in lead-poisoned rats, which was significant at a dose of 500 ppm lead ($P<0.01$, Figures 1C and 1D).

As for the spatial memory, exposing with a dose of 500 ppm lead led to decrease in both time and distance that the rats swam in the target quadrant ($P<0.05$ or $P<0.01$), either in 1 or 2 months (Figures 1E–1H). Memory disturbance was also observed in animals that had received lead 100 ppm for 2 months ($P<0.01$), BBR effectively improved memory in lead-poisoned rats ($P<0.05$ or $P<0.01$, Figures 1G and 1H).

Activity of Antioxidant Enzymes CAT, SOD and GPX in Hippocampus

The activity of hippocampal CAT, SOD and GPX did not change significantly in rats that exposed to lead for 1 month ($P>0.05$, Figures 2A–2C). But the activity of all enzymes decreased significantly in rats that received 100 and 500 ppm lead for 2 months ($P<0.05$ or $P<0.01$) and BBR could significantly improve the activity of enzymes ($P<0.05$ or $P<0.01$, Figures 2D–2F).

Hippocampal MDA Level

A high dose of lead (500 ppm) increased the level of MDA even in rats that were exposed to this dose for 1 month ($P<0.05$, Figures 3A and 3B). Low dose of lead (100 ppm) increased MDA level after 2 months ($P<0.05$). The amount of MDA in lead-

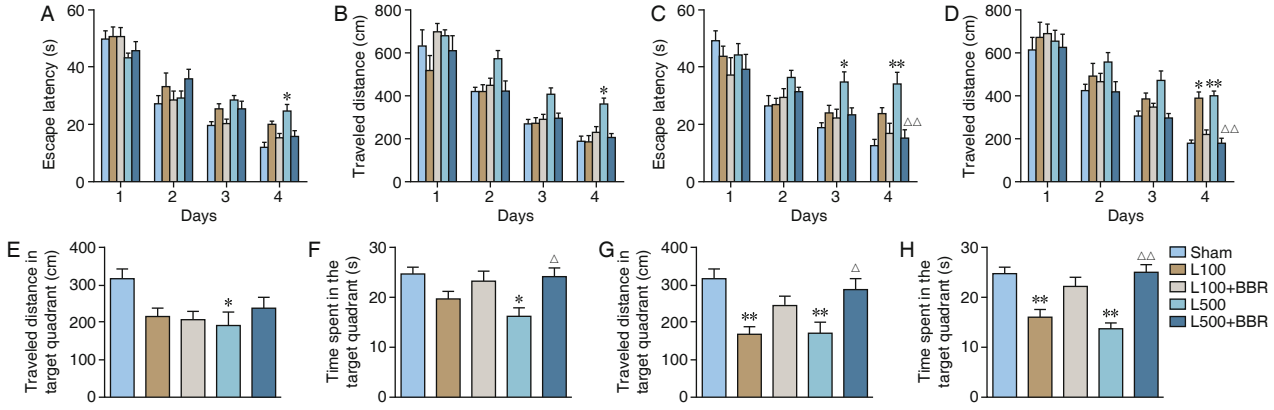

Figure 1. Spatial Learning and Memory by MWM Test ($\bar{x} \pm SE$, $n=10$)

Notes: Time and distance to reach the platform (learning assessment) in rats exposed to lead for 1 (A, B) and 2 months (C, D). Time spent and distance traveled in the target quadrant (memory assessment) in rats exposed to lead for 1 (E, F) and 2 months (G, H). $^{*}P<0.05$, $^{**}P<0.01$ *vs*. sham group; $^{\triangle}P<0.05$, $^{\triangle\triangle}P<0.01$ *vs*. same dose of lead group. L100: lead 100 ppm; L500: lead 500 ppm; BBR: berberine hydrochloride; MWM: Morris Water Maze test; $\bar{x} \pm SE$: mean ± standard error; the same below

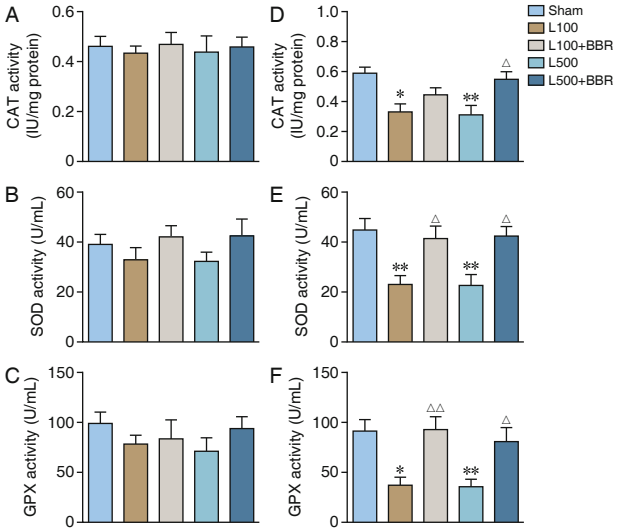

Figure 2. Activity of Hippocampus CAT, SOD, GPX ($\bar{x} \pm$SE, n=6)

Notes: Activity of CAT, SOD and GPX enzymes in hippocampus of rats exposed to lead for 1 (A–C) and 2 months (D–F). $^{*}P$<0.05, $^{**}P$<0.01 *vs*. sham group; $^{\triangle}P$<0.05, $^{\triangle\triangle}P$<0.01 *vs*. same dose of lead group. CAT: catalase; SOD: superoxide dismutase; GPX: glutathione peroxidase

poisoned rats treated with BBR showed a significant decrease at 2 months (P<0.05, Figure 3B).

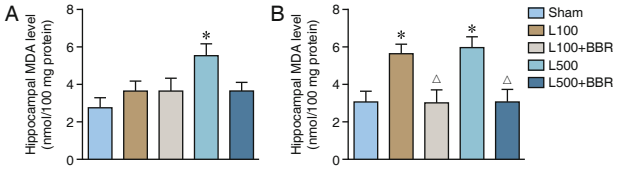

Figure 3. Hippocampal MDA Level ($\bar{x} \pm$SE, n=6)

Notes: Rats exposed to lead for 1 (A) and 2 months (B). $^{*}P$<0.05 *vs*. sham group; $^{\triangle}P$<0.05 *vs*. same dose of lead group. MDA: malondialdehyde

Histological Assessment of Hippocampus by HE Staining

The number of necrotic neurons, which have a wrinkled and dark appearance, increased in the hippocampal CA1 subregion in lead-poisoned rats. In the lead groups treated with BBR, the number of necrotic bodies decreased (Figure 4A). The density of healthy cells in the CA1 area decreased in rats that exposed to lead 500 ppm (P<0.05 or P<0.01) and BBR increased the density of healthy cells in a favorable form (P<0.05). Lead 100 ppm had no significant effect on cell density (P>0.05, Figures 4B and 4C).

DISCUSSION

The present study investigated the effect of BBR on subchronic and chronic lead poisoning with doses of 100 and 500 ppm lead acetate on the hippocampus in rats. One of the important target organs of lead is the nervous system.[2,3] High amounts of lead cause neurotoxicity which involves the central and peripheral nervous systems. Advanced degeneration of part of the brain is common in children exposed to high lead levels. High amounts of lead cause severe neurological symptoms such as paralysis, convulsions, coma, and ataxia.[3,12] Low amounts of lead can cause hyperactivity, irritability, and lack of attention and concentration.[2,13] Conversely, high amounts of lead may cause a decrease in the percentage of alertness, growth disorders, hearing loss, and memory and learning disorder.[12,14]

The results mentioned above are consistent with the results of the present study, which showed a high dose of lead, even in the short term, caused cognitive impairment, hippocampal tissue damage, and increased hippocampal lipid peroxidation. In the investigation of antioxidant activity, these results were obtained that short-term poisoning with lead, even a high dose of lead, did not change the antioxidant activity. This issue can probably be due to the antioxidant reserves have been relatively reduced and the other hand, these low

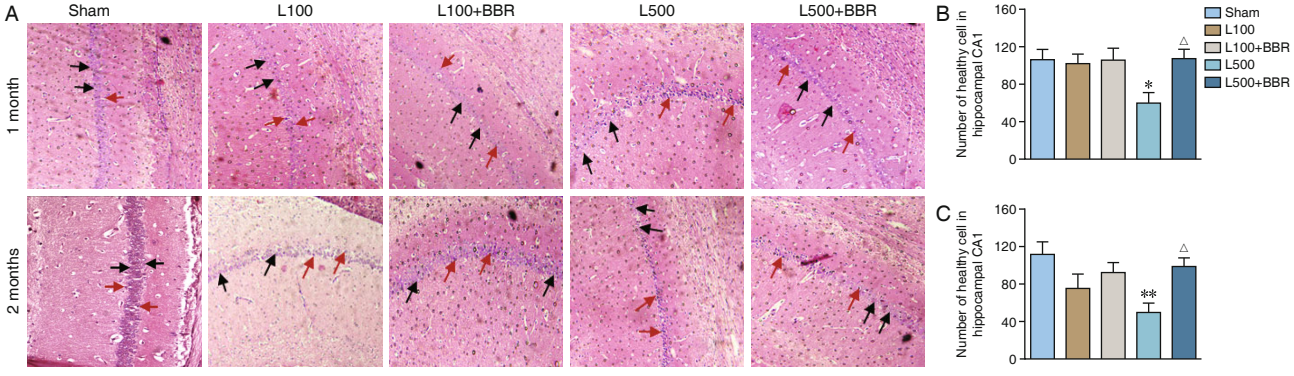

Figure 4. Number of Necrotic and Healthy Cells in Hippocampal CA1 Region by HE Staining ($\bar{x} \pm$SE, n=4)

Notes: (A) HE staining (×400). The black arrows indicate intact cells and red arrows indicate necrotic cells. (B, C) Rats exposed to lead for 1 and 2 months, respectively. $^{*}P$<0.05, $^{**}P$<0.01 *vs*. sham group; $^{\triangle}P$<0.05 *vs*. same dose of lead group. HE: hematoxylin and eosin

antioxidant reserves have become more active.[15] Finally, it may cause the final activity of antioxidants not to change in the short term. However, even with a low dose of lead, the activity of antioxidants decreased during chronic poisoning, which may be the result of reducing the antioxidant reserves of the hippocampus tissue in response to long-term lead poisoning.[16] Considering that lead has a meager elimination rate in the human body, even small amounts of lead have harmful effects on the body in the long term. Administration of lead in the long term causes DNA fragmentation and apoptosis in the frontal cortex, hippocampus and cerebellum.[15,17] Hippocampal destruction may be responsible for the clinical features of lead poisoning, such as impairments in emotional response, memory, and learning. In the central nervous system, lead can interfere with the action of the neurotransmitter glutamate, which is related to neural development. Lead affects synaptic function mainly by disrupting N-methyl-D-aspartate glutamate receptors, which are responsible for synaptic plasticity.[15,18] Moreover, the release of dopamine and the activity of the brain cholinesterase enzyme are inhibited by lead, which can be related to changes in motor skills and excessive excitability behavior.[19] Lead specifically increases the level of oxidative stress. Lead is attached to the mitochondrial membranes, penetrates the space of the mitochondrial matrix, and prevents oxidative phosphorylation and electron transfer in the mitochondria of neurons, ultimately increasing oxidative stress.[20,21]

Considering the importance of oxidative stress in lead-induced neurotoxicity, antioxidative factors can probably effectively reduce the adverse effects of lead on the nervous system. In this study, BBR was used as a known antioxidant. The results obtained from the effect of BBR on the changes caused by lead in the present study showed that BBR reduced tissue damage and lipid peroxidation in the hippocampus of rats exposed to lead, which leads to the improvement of the cognitive function of the hippocampus. Similarly, BBR improved the antioxidant activity of the hippocampus of lead-poisoned rats.

BBR has been used to treat various diseases, including diabetes, metabolic syndrome, fatty liver disease, and coronary heart disease.[22,23] Its protective effects on the heart have been widely reported.[24,25] Many studies have proven the beneficial antioxidant function of BBR.[9,26] The effect of BBR as an effective factor on the activity of antioxidants in various neurological disorders has also been confirmed.[27] A study by Adefegha, et al[28] has been showed that BBR exerts its protective effects due to its antioxidant properties and through the preservation of antioxidant enzymes in the brain of streptozotocin-induced diabetic Wistar rats. Several studies have shown the effect of BBR in reducing cognitive disorders. Treatment of diabetic animals with BBR reduced cognitive impairment in animals.[29] A review showed that BBR increases the activity of antioxidants, reduces inflammatory factors, increases neurotransmitters such as norepinephrine and serotonin, and increases neuromodulators such as vascular growth factor, which can reduce cognitive disorders following anxiety and depression.[30] In the model of chronic cerebral ischemia, BBR reduced tissue damage by increasing the activity of antioxidants, reducing apoptosis, and finally led to the improvement of the cognitive function of the animals.[9,11] BBR has also improved cognitive ability in the elderly with advanced dementia, accompanied by increased blood Bcl-2 levels and decreased serum levels of tumor necrosis factor alpha.[27]

In conclusion, the present study showed that BBR was able to reduce the oxidative damage of the hippocampus and improve the learning and memory of rats by increasing the activity of antioxidants and reducing oxidative stress.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Author Contributions

FZM and ASR actively participated in design, data analysis and collection, and writing of the article. FSZ, ZH, RM and FAA contributed in the data analysis and collection of the article. All the authors are fully aware of the final version of article. Article submission is done with the consent of all authors.

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(Accepted March 13, 2023)
Edited by YU Ming-zhu