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Original article

Harmine Mitigates Liver Injury Induced by Mercuric Chloride via the Inhibition of Oxidative Stress

Cyrus Jalili[1], Sara Darakhshan[2], Mohammadreza Azimi[3], Ali Ghanbari[1*]

[1]Department of Anatomy, Medical School, Kermanshah University of Medical Sciences, Kermanshah, Iran.
[2] Pharmaceutical Sciences Research Center, Health Institute, Kermanshah University of Medical Sciences, Kermanshah, Iran.
[3]Department of Biochemistry, Medical Faculty, Saveh Branch, Islamic Azad University, Saveh, Iran.

Abstract

Background and objective: The mercury-induced liver pathogenesis is mainly mediated by oxidative stress. The aim of the current study was to evaluate the possible ameliorative effect of harmine, a natural compound, on liver toxicity induced by mercury chloride ($HgCl_2$). **Methods:** Forty-two male Balb/c mice were randomly divided into six groups (n = 7): Control, $HgCl_2$ (0.5 mg/kg), harmine (20 mg/kg), and $HgCl_2$ (0.5 mg/kg) + harmine (5, 10, or 20 mg/kg). The mice received treatments once per day for two weeks. After this period, the blood and tissue samples were collected for analyses. **Results:** $HgCl_2$ caused a significant increase in levels of hepatic enzymes alanine aminotransferase, aspartate transaminase, and alkaline phosphatase; while harmine ameliorated these effects. Harmine in $HgCl_2$-intoxicated mice, showed protective effects as evidenced by the increase in liver relative weight to body as well as the diameter of central vein in the co-treated group. Serum levels of malondialdehyde and nitric oxide increased in $HgCl_2$, while they were declined in harmine co-treated groups compared to $HgCl_2$ group. The serum level of superoxide dismutase and total antioxidant capacity improved following harmine treatment in the co-administrated group compared to $HgCl_2$ group. Moreover, gene expression analysis demonstrated that harmine treatment improved the $HgCl_2$-induced decreasing of Ho-1, Nrf2, Hqo1, and Trx1. The histopathological examination confirmed the protective effects of harmine. **Conclusion**: Mercury can induce toxicity by elevation of oxidative stress in the liver and harmine attenuates hepatic injury induced by $HgCl_2$, at least in part, through its antioxidant activities.

Keywords: harmine; hepatotoxicity; liver; mercuric chloride; oxidative stress

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Introduction

Mercury (Hg), a heavy metal, is a ubiquitous pollutant with toxic effects in any form of life. It is derived from natural sources and human activities and is easily distributed in air, water, and soil [1]. Since mercury is abundant in the environment, it is nearly impossible for most people to avoid its exposure; hence, poisoning from occupational exposure and environmental pollution continues to be a concern [2]. High levels of Hg exposure cause toxic effects through progressive irreversible accumulation in tissues and organs, including the nerves, cardiovascular, reproductive and hepatic tissues, and the immune system [3,4]. The liver is the major site for metabolism and detoxification, and previous studies have shown that the liver is the mostly affected organ by mercury exposure [5].

A central mechanism of hepatic toxicity caused

*Corresponding author: aghanbari@kums.ac.ir

by mercuric chloride ($HgCl_2$) is associated with the oxidative stress [5-8]. Previous studies have shown that $HgCl_2$ inhibits the activities of free radical scavenging systems in the liver. It is found that $HgCl_2$ elevates biochemical parameters of the liver such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), gamma glutamyl transferase (GGT), and bilirubin, while the level of total protein is significantly declined [7]. It has also suppressed the antioxidant defense system such as activities of catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD) and has increased the hepatic malondialdehyde (MDA) concentration [9].

In recent years, the use of natural antioxidants in the treatment of heavy metal poisoning has become widespread [10]. Harmine as one of the active plant-derived compounds, is a tricyclic alkaloid. It possesses a wide range of ameliorative/protective properties, and studies confirmed that harmine has antioxidant and anti-inflammatory activities in some organs [11-13]. A study displayed harmine could mitigate lipopolysaccharide (LPS)-induced acute kidney injury through inhibition of oxidative stress and inflammation [12]. In another study, harmine showed beneficial effects on nicotine-induced liver failure in mice the prevention of oxidative stress [13]. Considering harmine role in oxidative stress and inflammatory states, it is assumed that this compound can ameliorate the oxidative stress caused by $HgCl_2$ in hepatic tissue. Hence, the aim of this work was to explore the potential protective effects of harmine on $HgCl_2$-induced liver injury and its underlying mechanisms in regulating oxidative stress.

Materials and Methods

Ethical considerations

The study protocol was approved by the Ethics Committee of Kermanshah University of Medical Sciences (IR.KUMS.REC.1398.945; June 2019) and the animal care was in accordance with the related guidelines.

Chemicals

Harmine (7-Methoxy-1-methyl-9H-pyrido[3,4-b] indole) powder was purchased from Sigma, USA (CAS No: 442-51-3). The levels of MDA and SOD were determined by commercial kits from ZellBio (GmbH, Germany). Total antioxidant capacity (TAC) was measured by Total Antioxidant Status kit (TAS, RANDOX Reagents, UK). The ALT, ALP, and AST serum levels were detected using related Pars Azmoon kits (Iran) according to the manufacturer's instructions. Nitric oxide (NO) level was evaluated using Griess reagent (Griess Reagent System, Promega; USA).

Animals and experimental protocol

In this study, forty-two male Balb/c mice (27-30 g) were housed in laboratory conditions (12 h of lightness/12 h of darkness, 21-23 °C) with ad libitum access to water and food. The animals were randomly divided into six groups (n = 7): Control (vehicle, saline only); $HgCl_2$ (0.5 mg/kg) + vehicle; vehicle + harmine (20 mg/kg); and $HgCl_2$ (0.5 mg/kg) + harmine (5, 10, or 20 mg/kg).

For randomization, we applied the completely randomized (between subjects) design. In this design, one of the groups is considered as control, and the animals are assigned to the treatment groups strictly at random. Besides, the positions within the animal house and the order in which the treatments are given and the outcome is measured should also be in random order. Harmine was administrated one hour later than $HgCl_2$ treatment. The doses of $HgCl_2$ and harmine were selected based on previous reports demonstrating the in vivo anti-inflammatory and antioxidant effects [14,15].

The mice received intraperitoneal saline, $HgCl_2$, and harmine once per day for two weeks. Twenty-four hours after the last treatment, all animals were anesthetized with a mixture of 70 mg/kg ketamine and 10 mg/kg xylazine (Alfasan, Holland). Blood samples were collected from the abdominal aorta into evacuated tubes and were immediately centrifuged at 3,000 rpm for 15 min at 4 °C to obtain the sera. For histological analysis, a portion of tissues was immersed in 10% buffered formalin, and a portion of samples was immediately frozen in liquid nitrogen and stored at −80 °C until use.

Biochemical analysis

Serum samples were assessed for the parameters related to oxidative stress including MDA, SOD, and total antioxidant capacity (Vagvala & O'Connor). The levels of MDA and SOD were determined by commercial kits (ZellBio GmbH, Germany) according to the manufacturer's instructions by a microplate reader (STAT FAX

2100, USA). TAC was measured by Total Antioxidant Status kit (TAS, RANDOX Reagents, UK) using a UV/VIS Spectrophotometer (UNICO SQ2800, USA). Alanine aminotransferase (ALT), alkaline phosphatase (ALP), and aspartate aminotransferase (AST) serum levels were detected using related Pars Azmoon kits according to the manufacturer's instructions. Nitric oxide (NO) level was evaluated using Griess reagent (Griess Reagent System, Promega) according to the manufacturer's instructions.

Histological examination

The liver tissues were fixed in buffered formalin 10% at 4 °C, embedded in paraffin, sectioned at 6-8 µm, and then processed for hematoxylin and eosin (H&E) staining. After staining, images were captured using a light microscope (Olympus CH3, Japan). To evaluate the possible variation following treatments, the diameter of one-hundred central vein was measured using DP2-BSW software associated to the microscope in random fields and averaged.

RNA isolation and qRT-PCR

Liver tissues were taken from the animals and stored in liquid nitrogen until processed for RNA isolation. Total RNA was extracted from all groups using a standard RNA extraction protocol (TRIzol reagent, Invitrogen). RNA was reverse transcribed using PrimeScript First Strand cDNA Synthesis Kit (TaKaRa Bio). Quantitative RT-PCR (qRT-PCR) was performed using SYBR Green Master Mix (TaKaRa Bio Inc.) and Corbett Rotor-Gene 6000 thermocycler. Expression levels of target genes were normalized against beta-actin mRNA level, and the control group was expressed as 1 to indicate a precise fold change. Features of the primers are shown in Table 1.

Statistical analysis

Statistical analysis was performed using SPSS software (SPSS Inc., Chicago, IL, USA; version 19.0). The comparisons were performed using one-way ANOVA followed by Tukey's post-hoc and expressed as mean ± standard error of mean (SEM). A value of $p<0.05$ was considered as significant.

Results and Discussion

The notable role of oxidative stress in injury associated with mercuric chloride poisoning suggests that antioxidants may be beneficial to mitigate related toxicity [10,16-18]. We investigated the effect of harmine as a natural antioxidant on the $HgCl_2$-induced liver injury and the underlying elements involved in regulating oxidative stress. Studies established that mercury toxicity promotes the generation of reactive oxygen species (ROS) such as superoxide and hydrogen peroxides, which induce oxidative stress, resulting in lipid peroxidation and cell membrane damage of hepatocytes and activation of liver enzymes [15,19]. It is associated with binding to thiol groups in several proteins and peptides, a decrease in cellular antioxidants such as SOD, glutathione (GSH), and CAT, along with an increase in MDA [1,7,9,14,16,17,20]. In accordance with these results, as shown in Figure 1A, compared to the control group, serum MDA content was significantly increased after a 14-days period of $HgCl_2$ treatment ($p<0.001$), while adding harmine at 20 mg/kg as co-administration with $HgCl_2$ caused a reduction in its level ($p<0.01$). In the $HgCl_2$ group, SOD level sharply decreased ($p<0.001$); however, co-treatment with harmine resulted in an amelioration in generated toxicity (Figure 1B), which was significant at 20 mg/kg dose ($p<0.001$).

In this study, we also detected a significant decline in total antioxidant capacity in mice exposed to $HgCl_2$ ($p<0.001$).

Table 1. Primers used for qRT-PCR analysis

Gene	Sequence (5' to 3')
NAD(P)H:quinone oxidoreductases1 (Nqo1)	F: AAGGATGGAAGAAACGCCTGGAGA R: GGCCCACAGAAAGGCCAAATTTCT
Thioredoxin 1 (Trx1)	F: CCCTTCTTCCATTCCCTCT R: TCCACATCCACTTCAAGGAAC
Heme oxygenase-1 (Ho-1)	F: CCTTCCCGAACATCGACAGCC R: GCAGCTCCTCAAACAGCTCAA
Nuclear factor (erythroid-derived 2)-like 2 (Nrf2)	F: CAGCATGATGGACTTGGA R: TGAGACACTGGTCACACT
Actb	F: CACTTTCTACAATGAGCTGCG R: CTGGATGGCTACGTACATGG

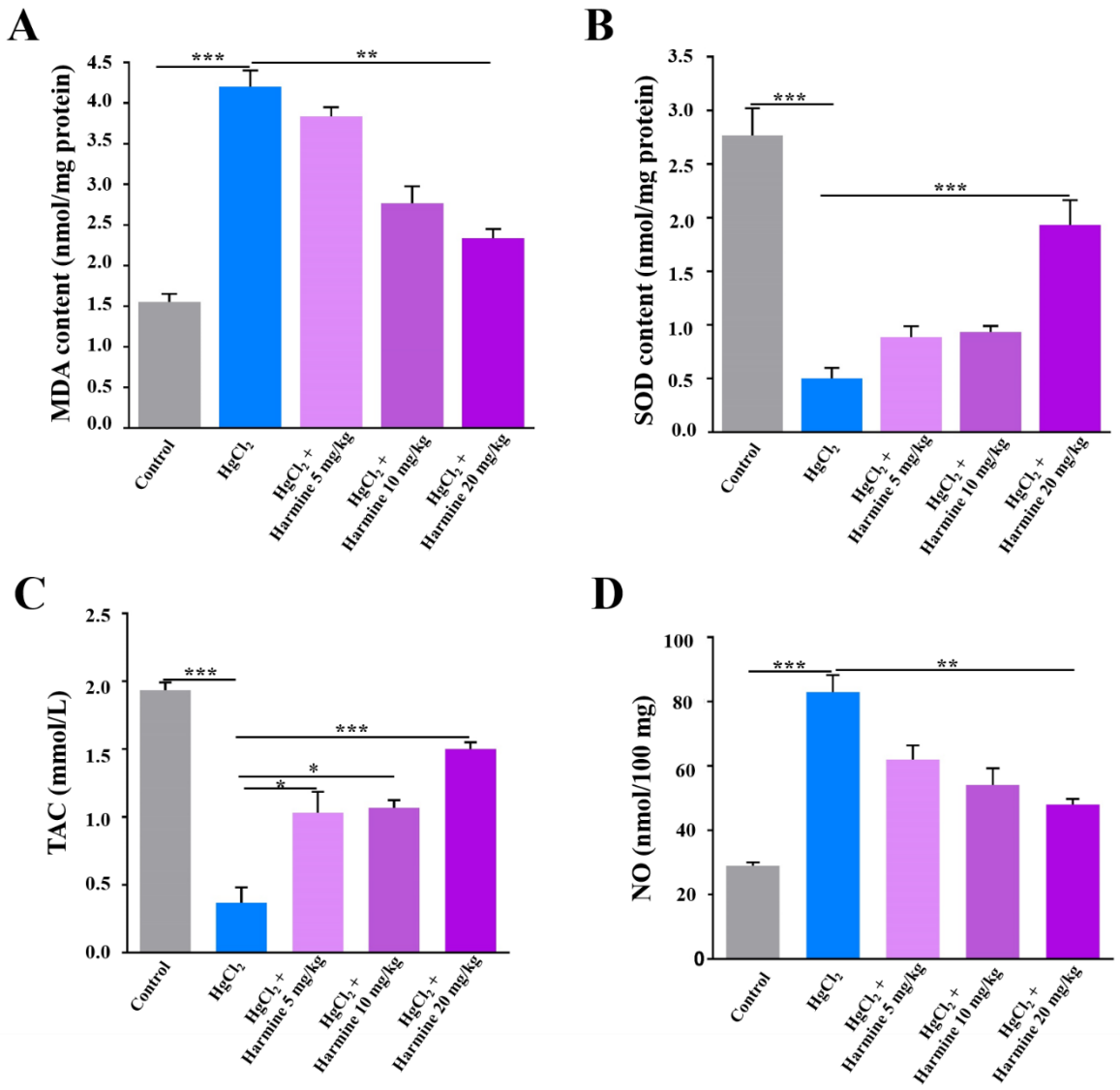

Figure 1. Effects of harmine on oxidative/nitrative stress in the $HgCl_2$-induced liver injury. Mice that received $HgCl_2$ (0.5 mg/kg) showed a significant increase in MDA (A) and NO (D) levels, also decrease in TAC (C) and SOD level (B), whereas treatment with harmine ameliorated these alterations. Data are expressed as mean ± SEM, n = 7. *p < 0.05, **p < 0.01, ***p < 0.001. TAC, total antioxidant capacity; MDA, malondialdehyde; SOD, superoxide dismutase; NO, nitric oxide

On the other hand, at all three doses used in this experiment, harmine showed an improvement in TAC index (Figure 1C). In LPS-induced kidney injury, pre-treatment with 25 or 50 mg/kg harmine markedly reduced the formation of MDA and myeloperoxidase (MPO), while it increased SOD and GSH activities [14].

Administration of $HgCl_2$ in mice resulted in a significant elevation in the serum levels of AST, ALT, and ALP as compared to the control group (Figure 2). Harmine at 20 mg/kg dose caused a significantly ($p<0.01$) decline in the level of AST in the $HgCl_2$ plus harmine treatment group. Also, harmine reduced the decreasing effect of $HgCl_2$ on ALT, significantly for both 10 and 20 mg/kg ($p<0.05$). Harmine also showed an ameliorative effect against nicotine-induced damage to the liver. Following nicotine administration, a major toxic component of cigarette smoke, AST, ALT, and ALP levels was elevated after a 28 days-period treatment. Also, harmine could improve thiobarbituric acid reactive species (TBARS), MDA, and NO levels in co-treated group. It can be concluded that harmine counteracted the oxidative stress of nicotine [21].

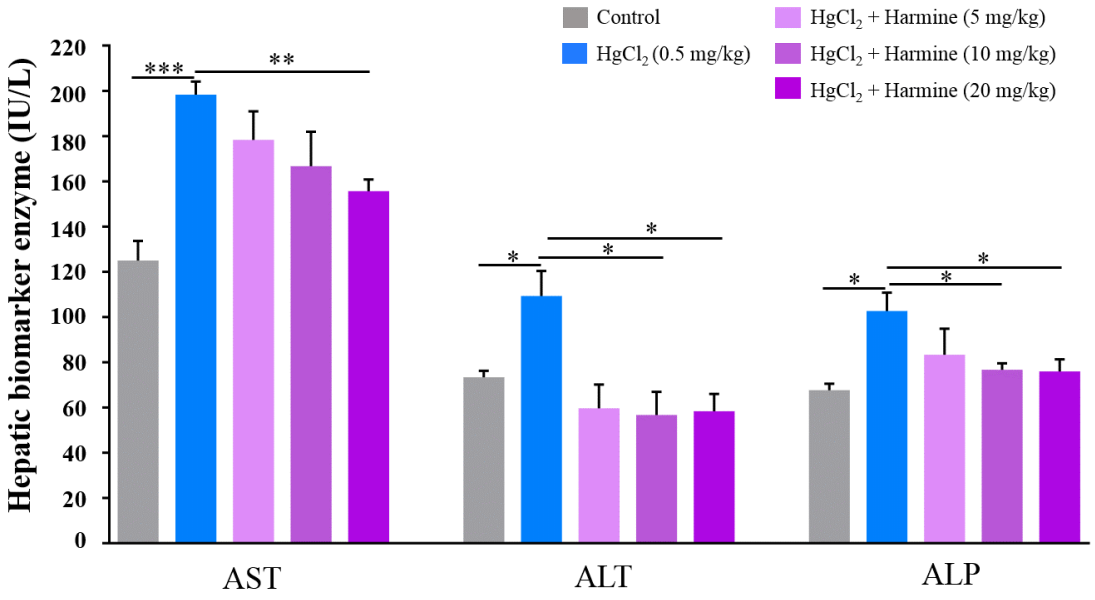

Figure 2. Effects of harmine on the liver injury biomarkers in $HgCl_2$-exposed mice. Data are expressed as mean ± SEM, n = 7; *p<0.05, **p<0.01 and ***p<0.001; AST: aspartate transaminase; ALT: alanine transaminase; ALP: alkaline phosphatase; $HgCl_2$: mercuric chloride

Elevations in ALT and AST is an important marker for hepatocellular damage, whereas, an elevation in ALP and bilirubin in disproportion to ALT and AST would denote a cholestatic pattern [22]. Animals in our study showed significant rises in AST and ALT enzymes in serum after $HgCl_2$ exposure. This could be due to the release of these enzymes from the cytoplasm, into the blood circulation after breaking the plasma membrane and hepatocyte injury [23]. Results obtained from a study by Joshi et al. showed that N-acetyl cysteine and selenium individually or in combination provided protection against mercury toxicity by decreasing AST, ALT, and LDH activities [23]. Alkaline phosphate (ALP) is a membrane protein and its alterations are likely to affect the membrane permeability and cause the imbalance in transport of metabolites. Moreover, Plaa et al. [24] have reported that this phosphate enzyme acts as an indicator of cholestatic changes. In our study, $HgCl_2$ administration to mice led to a marked elevation in the level of serum ALP (Figure 2) significantly for both 10 and 20 mg/kg ($p<0.05$), which is indicative of hepatocellular damage. In a study by Mumtaz et al., the effects of $HgCl_2$ administration and protective mechanism of ascorbic acid in heavy metals-induced hepatotoxicity was investigated in intoxicated rabbits. Supplementation of vitamin C as an antioxidant showed an ameliorating potential after 14 and 28 days by changing the alternations of ALAT, ASAT, LDH, GGT, and bilirubin parameters towards normal levels [7].

In addition, increased levels of inflammatory cytokines and NO and also impairment in the function of mitochondria are thought to complicate the harmful effects of mercury [25,26]. Biological defense produces both superoxide anion and NO during the oxidative stress triggered during inflammatory processes. Under these conditions, NO and the superoxide anion may react together to generate significant amounts of a much more active oxidative molecule, peroxynitrite anion ($ONOO^-$) [27]. In our study, compared to the control group, nitrosative stress was markedly increased in the $HgCl_2$-exposed mice as exhibited by a significant increase ($p<0.001$) in serum NO level. Its level, however, significantly decreased when 20 mg/kg harmine was added ($p<0.01$; Figure 1D). The increased serum NO level reflects inflammatory responses, whereas harmine showed anti-inflammatory effects by decreasing the secretion of this marker.

Our stereological and histopathological findings were consistent with each other. The weight of the liver decreased significantly in the $HgCl_2$-treated group compared to the liver of controls. Harmine at 20 mg/kg significantly improved the

weight of the liver compared to the related weight of $HgCl_2$-treated in co-administration group (Figure 3A; $p<0.05$). The diameter of the central vein increased significantly in the group exposed to Hg compared to controls ($p<0.01$), and harmine at 20 mg/kg could ameliorate this effect of $HgCl_2$ (Figure 3B; $p<0.05$). The liver of the control group appeared normal (Figure 3C). We observed enlarged blood vessels and dilated sinusoids with increased perivascular connective tissue in the $HgCl_2$-treated group. The volume of the parenchyma decreased, but the volume of the sinusoids increased in the $HgCl_2$-treated group compared to controls (Figure 3D). On the other hand, the density and total number of hepatocytes reduced in the group exposed to $HgCl_2$, which was resulted from the degeneration and death of these cells. The harmine-treated group (at 20 mg/kg dose) was in a similar situation to the control group (Figure 3E). However, in co-administration group, the signs of improvements towards the control group were shown. The density and total number of hepatocytes improved in the harmine co-administrated group (Figure 3F).

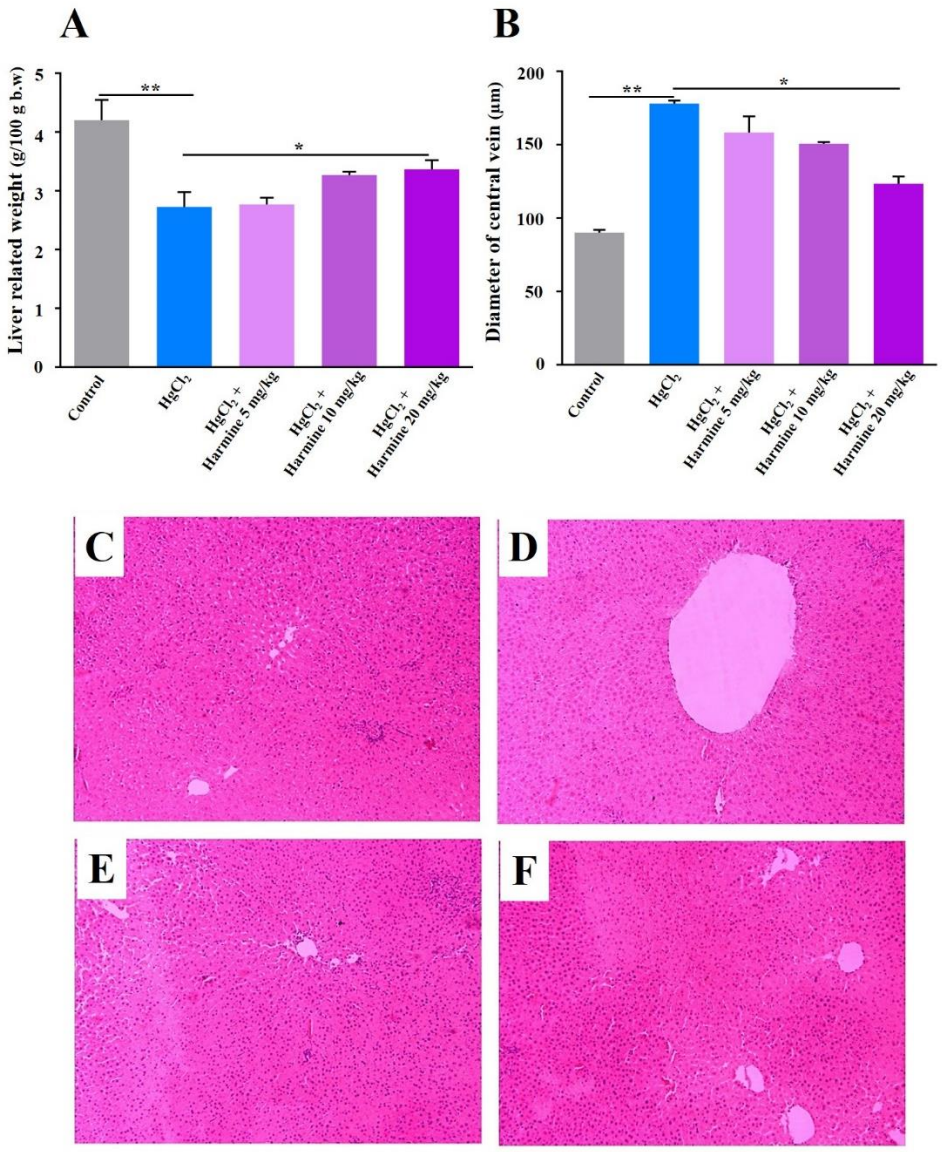

Figure 3. Effects of harmine on histopathological changes in the $HgCl_2$-induced hepatic damage. A) Liver relative weight to the body weight, and B) diameter (µm) of the central vein. H&E staining of the liver of C) control group showing the normal structure of tissue including parenchymal and non-parenchymal cells; D) $HgCl_2$-administrated mice showing enlarged blood vessels and dilated sinusoids with increased perivascular connective tissue. E) The harmine-treated group (at 20 mg/kg dose) was in a similar situation to the control group. F) Administration of harmine at 20 mg/kg dose protected hepatic tissue against histopathological alterations induced by $HgCl_2$.

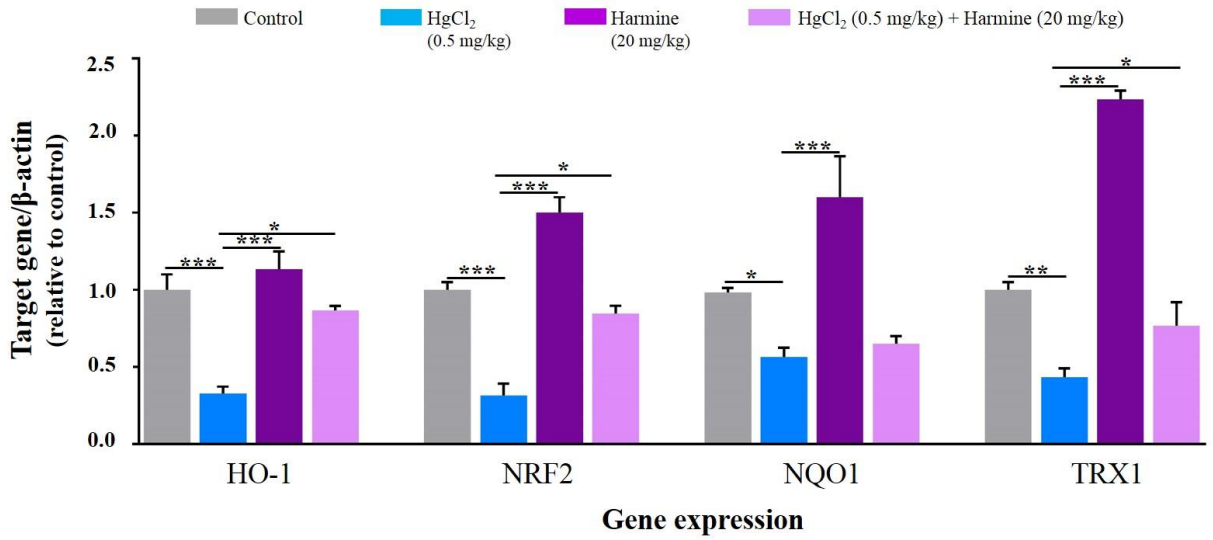

Figure 4. Effects of harmine on the expression of genes involved in oxidative stress in the liver of $HgCl_2$-induced injury. Administration of $HgCl_2$ significantly decreased mRNA level of Ho-1, Nrf2, Nqo1, and Trx1. Data are expressed as mean ± SEM, n = 7. *p < 0.05, **p < 0.01, and ***p < 0.001. HO-1, heme oxygenase-1; NRF2, nuclear factor erythroid 2-related factor 2; NQO1, NAD(P)H quinone dehydrogenase 1; TRX1, thioredoxin 1. The expression of transcripts was normalized against beta-actin, where the control group was expressed as 1 to indicate a precise fold change.

The nuclear height of the hepatocytes significantly decreased in the Hg exposed group and the nuclear diameter of hepatocytes significantly decreased. We believe that the decrease might be due to alteration of the genetic material including pyknotic or heterochromatic changes [28].

ROS levels have been shown to influence the expression of key genes involved in regulating cellular and systemic oxidative stress [29]. The effects of $HgCl_2$ and harmine on the mRNA level of thioredoxin (Trx), nuclear factor erythroid 2-related factor 2 (Nrf2), heme oxygenase-1 (HO-1), and (NAD(P)H quinone dehydrogenase 1 (Nqo1) evaluated by qRT-PCR (Figure 4). The expression of all genes significantly declined following $HgCl_2$ exposure in the liver of exposed mice ($p<0.05$). Thioredoxin system consists of thioredoxin (Trx) and thioredoxin reductase (TrxR) senses and responds to oxidative stress and modulates the redox status by scavenging ROS and by regulating several signaling proteins [30]. Trx1 functions as a molecular switch turning the cellular redox state into kinase signaling; thus, it is able to regulate DNA synthesis, cell proliferation, apoptosis, and transcription [31,32]. Trx1 itself is regulated by oxidative stress conditions via binding of NRF2 to an antioxidant responsive element (ARE) in the Trx promotor [33]. We observed that the expression of Trx1 increased by harmine treatment. Also, harmine plus $HgCl_2$ improved the decreased level of Trx1. We observed that Trx1 gene expression significantly increased following harmine treatment ($p<0.001$). In addition, compared with $HgCl_2$ group, expression of Trx1 significantly increased in the co-administrated group ($p<0.05$).

Compared with 0.5 mg/kg $HgCl_2$ group, the Nrf2 gene expression significantly increased in harmine-only treatment group and 20 mg/kg harmine plus $HgCl_2$ group, $p<0.001$ and $p<0.05$, respectively.

Nrf2 is a transcription factor that is upregulated in response to oxidative stress and regulates transcription of genes encoding for cytoprotective enzymes and other proteins crucial for maintaining cellular homeostasis [29]. During oxidative stress, Nrf2 translocates to the nucleus and activates transcription of target genes such as NQO-1 and HO-1 [34]. Increased levels of oxidative stress caused by $HgCl_2$ might be attributed to insufficient ROS scavenging because of a failure in Nrf2 activation. However, we found that harmine could activate this antioxidant pathway, at the mRNA level.

A number of stimuli like nitric oxide, heavy metals, cytokines, and modified lipids have been shown to induce HO-1 expression [35]. The activation of HO-1 appears to be an endogenous defensive mechanism applied by cells to reduce inflammation and tissue damage in hepatic

chemically induced injury [36]. Our results showed that harmine could ameliorate the negative effect of $HgCl_2$ on Ho-1 expression in liver tissue. Harmine administration alone increased the expression level of this gene.
Combined treatment of harmine by $HgCl_2$ exposure exhibited a significant increase ($p<0.05$) in Ho-1 when compared with Hg-treated group. also, harmine significantly improved the expression of Ho-1 ($p<0.001$).
The expression of Nqo1 gene in human and mouse are primarily regulated via ARE sequences in the promoter region, which controls redox homeostasis and facilitates adaptation of most cells to oxidative stress [37]. Nqo1 mRNA and protein expression and enzyme activity in mouse livers increased after bile duct ligation in wild-type mice, but not in Nrf2-null mice, a state that caused hepatocellular oxidative stress and injury [38]. A study on Nqo1-knockout mice revealed that this gene has a protective effect in acetaminophen-induced hepatotoxicity. In the absence of the role of Nqo1, acetaminophen triggered cell death in hepatocytes, severe mitochondrial dysfunction, and oxidative stress [39]. Previous studies have shown harmine could reduce oxidative stress, inflammation responses, and also apoptosis through suppressing the expression of nuclear factor kappaB (NF-κB), interleukin-1β (IL-1β), and caspase-1 [12,14]. In our study, Nqo1 gene expression significantly increased in 20 mg/kg harmine group ($p<0.001$). As expected, harmine-only resulted in a significant increase in Nqo1 expression level. The expression of Nqo1 was not significantly different for the co-administrated group compared to $HgCl_2$ group.
Although massive efforts are done in search for novel therapeutics against the mercury-induced toxicity, there is no effective treatment that completely eliminates the toxic effects [40]. Accordingly, the use of compounds having antioxidant properties and few apparent side effects may represent a helpful adjuvant strategy to eliminate the toxicity caused by organic and inorganic mercury forms [16,19,23,41,42].

Conclusion

In conclusion, the results of this study suggested that harmine has the potential to protect against mercury-induced hepatotoxicity. In addition, the mechanism of protection by harmine may be through modifying the enzymatic and non-enzymatic antioxidant levels. Based on these findings, it can be concluded that harmine can be a promising candidate for the management of liver mercury-intoxication.

Acknowledgments

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Author contributions

Cyrus Jalili was involved in conceptualization, data curation, validation and funding acquisition; Sara Darakhshan performed the analysis and prepared the original draft of the manuscript; Mohammadreza Azimi contributed in investigations, writing and editing of the manuscript;Ali Ghanbari contributed in project administration, supervision, writing and editing of the manuscript, validation and funding acquisition.

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

References

[1] Patel TA, Rao MV. Ameliorative effect of certain antioxidants against mercury induced genotoxicity in peripheral blood lymphocytes. *Drug Chem Toxicol*. 2015; 38(4): 408–414.
[2] Bridges CC, Joshee L, Zalups RK. Aging and the disposition and toxicity of mercury in rats. *Exp Gerontol*. 2014; 53: 31–39.
[3] Fernandes Azevedo B, Barros Furieri L, Peçanha FM, Wiggers GA, Frizera Vassallo P, Ronacher Simões M, Fiorim J, De Batista PR, Fioresi M, Rossoni L, Stefanon I, Alonso MJ, Salaices M, Vassallo DV. Toxic effects of mercury on the cardiovascular and central nervous systems. *Biomed Res Int*. 2012; Article ID 949048.
[4] Mahaffey KR. Mercury exposure: medical and public health issues. *Trans Am Clin Climatol Assoc*. 2005; 116: 127–153.
[5] Lee MR, Lim YH, Lee BE, Hong YC. Blood mercury concentrations are associated with decline in liver function in an elderly population: a panel study. *Environ Health*. 2017; 16(1): 1–8.
[6] Choi J, Bae S, Lim H, Lim JA, Lee YH, Ha M, Kwon HJ. Mercury exposure in association with decrease of liver function in

adults: a longitudinal study. *J Prev Med Public Health*. 2017; 50(6): 377–385.

[7] Mumtaz S, Ali S, Khan R, Andleeb S, Ulhaq M, Khan MA, Abdullah Shakir H. The protective role of ascorbic acid in the hepatotoxicity of cadmium and mercury in rabbits. *Environ Sci Pollut Res Int*. 2019; 26(14): 14087–14096.

[8] Necib Y, Bahi A, Zerizer S. Amelioration of mercuric chloride toxicity on rat liver with Argan oil and sodium selenite supplements. *Int J Pharm Bio Sci*. 2013; 4(2): 839–849.

[9] Zeng L, Zheng JL, Wang YH, Xu MY, Zhu AY, Wu CW. The role of Nrf2/Keap1 signaling in inorganic mercury induced oxidative stress in the liver of large yellow croaker *Pseudosciaena crocea*. *Ecotoxicol Environ Saf*. 2016; 132: 345–352.

[10] GarcíaNiño WR, PedrazaChaverrí J. Protective effect of curcumin against heavy metalsinduced liver damage. *Food Chem Toxicol*. 2014; 69: 182–201.

[11] Hamsa T, Kuttan G. Harmine inhibits tumour specific neovessel formation by regulating VEGF, MMP, TIMP and proinflammatory mediators both in vivo and in vitro. *Eur J Pharmacol*. 2010; 649(13): 64–73.

[12] Liu X, Li M, Tan S, Wang C, Fan S, Huang C. Harmine is an inflammatory inhibitor through the suppression of NFκB signaling. *Biochem Biophys Res Commun*. 2017; 489(3): 332–338.

[13] Salahshoor MR, Roshankhah S, Motavalian V, Jalili C. Effect of harmine on nicotineinduced kidney dysfunction in male mice. *Int J Prev Med*. 2019; Article ID 31360344.

[14] Niu X, Yao Q, Li W, Zang L, Li W, Zhao J, Liu F, Zhi W. Harmine mitigates LPS-induced acute kidney injury through inhibition of the TLR4NFκB/NLRP3 inflammasome signalling pathway in mice. *Eur J Pharmacol*. 2019; 849: 160–169.

[15] Hussain S, Atkinson A, Thompson S, Khan A. Accumulation of mercury and its effect on antioxidant enzymes in brain, liver, and kidneys of mice. *J Environ Sci Health B*. 1999; 34(4): 645–660.

[16] da Luz Fiuza T, Leitemperger J, Severo ES, Marins AT, do Amaral AB, Pereira ME, Loro VL. Effects of diphenyl diselenide diet on a model of mercury poisoning. *Mol Biol Rep*. 2018; 45(6): 2631–2639.

[17] Caglayan C, Kandemir FM, Darendelioğlu E, Yıldırım S, Kucukler S, Dortbudak MB. Rutin ameliorates mercuric chlorideinduced hepatotoxicity in rats via interfering with oxidative stress, inflammation and apoptosis. *J Trace Elem Med Biol*. 2019; 56: 60–68.

[18] Ma Y, Zheng Y, Dong X, Zou X. Effect of mercury chloride on oxidative stress and nuclear factor erythroid 2-related factor 2 signalling molecule in liver and kidney of laying hens. *J Anim Physiol Anim Nutr (Berl)*. 2018; 102(5): 1199–1209.

[19] Jalili C, Roshankhah S, Jalali A, Salahshoor MR. Hepatoprotective activity of royal jelly on mercuric chloride–induced damage model in rats. *J Rep Pharma Sci*. 2019; 8(2): 181-187.

[20] Lu SC. Glutathione synthesis. *Biochim Biophys Acta*. 2013; 1830(5): 3143–3153.

[21] Salahshoor MR, Mahmoudian ZG, Roshankhah S, Farokhi M, Jalili C. Harmine shows therapeutic activity on nicotine-induced liver failure in mice. *Histol Histopathol*. 2019; 34(10): 1185–1193.

[22] Vagvala SH, O'Connor SD. Imaging of abnormal liver function tests. *Clin Liver Dis (Hoboken)*. 2018; 11(5): 128–134.

[23] Joshi D, Mittal DK, Shukla S, Srivastav AK, Srivastav SK. Nacetyl cysteine and selenium protects mercuric chlorideinduced oxidative stress and antioxidant defense system in liver and kidney of rats: a histopathological approach. *J Trace Elem Med Biol*. 2014; 28(2): 218–226.

[24] Plaa GL. Detection and evaluation of chemically induced liver injury. In: Hayes W Ed. Principles and methods of toxicology. New York: Raven Press, 1982.

[25] Ahmad S, Mahmood R. Mercury chloride toxicity in human erythrocytes: enhanced generation of ROS and RNS, hemoglobin oxidation, impaired antioxidant power, and inhibition of plasma membrane redox system. *Environ Sci Pollut Res Int*. 2019; 26(6): 5645–5657.

[26] Belyaeva EA, Korotkov SM, Saris NE. In vitro modulation of heavy metalinduced rat liver mitochondria dysfunction: a comparison of copper and mercury with cadmium. *J Trace Elem Med Biol*. 2011; 25(S1): 63–73.

[27] Jomova K, Valko M. Advances in metalinduced oxidative stress and human disease. *Toxicology*. 2011; 283(23): 65–87.
[28] Chen C, Qu L, Li B, Xing L, Jia G, Wang T, Gao Y, Zhang P, Li M, Chen W, Chai Z. Increased oxidative DNA damage, as assessed by urinary 8-hydroxy-2′ deoxyguanosine concentrations, and serum redox status in persons exposed to mercury. *Clin Chem*. 2005; 51(4): 759–767.
[29] Amadi CN, Offor SJ, Frazzoli C, Orisakwe OE. Natural antidotes and management of metal toxicity. *Environ Sci Pollut Res Int*. 2019; 26(18): 18032–18052.
[30] Ahsan MK, Lekli I, Ray D, Yodoi J, Das DK. Redox regulation of cell survival by the thioredoxin superfamily: an implication of redox gene therapy in the heart. *Antioxid Redox Signal*. 2009; 11(11): 2741–2758.
[31] Prigge JR, Eriksson S, Iverson SV, Meade TA, Capecchi MR, Arnér ES, Arnér ES, Schmidt EE. Hepatocyte DNA replication in growing liver requires either glutathione or a single allele of txnrd1. *Free Radic Biol Med*. 2012; 52(4): 803–810.
[32] Song JS, Cho HH, Lee BJ, Bae YC, Jung JS. Role of thioredoxin 1 and thioredoxin 2 on proliferation of human adipose tissue-derived mesenchymal stem cells. *Stem Cells Dev*. 2011; 20(9): 1529–1537.
[33] Kim YC, Masutani H, Yamaguchi Y, Itoh K, Yamamoto M, Yodoi J. Hemin-induced activation of the thioredoxin gene by Nrf2 a differential regulation of the antioxidant responsive element by a switch of its binding factors. *J Biol Chem*. 2001; 276(21): 18399–18406.
[34] Singh S, Vrishni S, Singh BK, Rahman I, Kakkar P. Nrf2ARE stress response mechanism: a control point in oxidative stress-mediated dysfunctions and chronic inflammatory diseases. *Free Radic Res*. 2010; 44(11): 1267–1288.
[35] Loboda A, Jazwa A, Grochot-Przeczek A, Rutkowski AJ, Cisowski J, Agarwal A, Jozkowicz A, Dulak J. Heme oxygenase1 and the vascular bed: from molecular mechanisms to therapeutic opportunities. *Antioxid Redox Signal*. 2008; 10(10): 1767–1812.
[36] Origassa CS, Câmara NO. Cytoprotective role of heme oxygenase1 and heme degradation derived end products in liver injury. *World J Hepatol*. 2013; 5(10): 541–549.
[37] Nioi P, McMahon M, Itoh K, Yamamoto M, Hayes JD. Identification of a novel Nrf2-regulated antioxidant response element (ARE) in the mouse NAD (P) H: quinone oxidoreductase 1 gene: reassessment of the ARE consensus sequence. *Biochem J*. 2003; 374(2): 337–348.
[38] Aleksunes LM, Slitt AL, Maher JM, Dieter MZ, Knight TR, Goedken M, Cherrington NJ, Chan JY, Klaassen CD, Manautou JE. Nuclear factorE2-related factor 2 expression in liver is critical for induction of NAD (P) H: quinone oxidoreductase 1 during cholestasis. *Cell Stress Chaperones*. 2006; 11(4): 356–363.
[39] Hwang JH, Kim YH, Noh JR, Gang GT, Kim KS, Chung HK, Tadi S, Yim YH, Shong M, Lee CH. The protective role of NAD (P) H: quinone oxidoreductase 1 on acetaminophen-induced liver injury is associated with prevention of adenosine triphosphate depletion and improvement of mitochondrial dysfunction. *Arch Toxicol*. 2015; 89(11): 2159–2166.
[40] Franco JL, Posser T, Missau F, Pizzolatti MG, Santos AR, Souza DO, Aschner M, Rocha JB, Dafre AL, Farina M. Structure–activity relationship of flavonoids derived from medicinal plants in preventing methylmercury-induced mitochondrial dysfunction. *Environ Toxicol Pharmacol*. 2010; 30(3): 272–278.
[41] Bharathi E, Jagadeesan G, Vijayakumar M. Hepatoameliorative effect of hesperidin and ellagic acid on mercuric chloride intoxicated rats. *Biomed Aging Pathol*. 2014; 4(1): 17–21.
[42] de Freitas AS, Funck VR, dos Santos Rotta M, Bohrer D, Mörschbächer V, Puntel RL, Nogueira CW, Farina M, Aschner M, Rocha JB. Diphenyl diselenide, a simple organoselenium compound, decreases methylmercury-induced cerebral, hepatic and renal oxidative stress and mercury deposition in adult mice. *Brain Res Bull*. 2009; 79(1): 77–84.

Abbreviations

ALP: Alkaline phosphate; ALT: alanine aminotransferase; AST: aspartate aminotransferase; CAT: catalase; GGT: gamma glutamyl transferase; GPx: glutathione

peroxidase; H&E: hematoxylin and eosin; HO-1: heme oxygenase-1; IL-1β: interleukin-1β; LDH: lactate dehydrogenase; LPS: lipopolysaccharide; MDA: malondialdehyde; NF-Kb: nuclear factor kappaB; NO: nitric oxide; Nqo1: NAD(P)H quinone dehydrogenase 1; Nrf2: nuclear factor erythroid 2-related factor 2; RNA, ribonucleic acid; qRT-PCR: quantitative reverse transcription polymerase chain reaction; SOD: superoxide dismutase; TAC: total antioxidant capacity; Trx: thioredoxin