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Research Article

Boldine Supplementation Regulates Mitochondrial Function and Oxidative Stress in a Rat Model of Hepatotoxicity

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ABSTRACT

Background: The xenobiotics-induced liver injury is a clinical complication. Hence, finding new hepatoprotective strategies has clinical value. Oxidative stress and its subsequent complications are major mechanisms involved in xenobiotics-induced hepatotoxicity. Boldine is one of the most potent antioxidant molecules widely investigated for its protective properties in different experimental models. In the current study, the hepatoprotective properties of boldine and its potential mechanisms of hepatoprotection have been investigated.

Methods: Rats received thioacetamide (TAA; 200 mg/kg, i.p) as a model of acute liver injury. Boldine (5, 10, 1nd 20 mg/kg; 24 hours intervals; oral) was administered as the hepatoprotective agent.

Results: Liver injury was evident in TAA-treated animals (48 hours after TAA exposure) as a severe increase in serum level of liver injury biomarkers and histopathological alterations. Moreover, markers of oxidative stress were increased in liver tissue of TAA-treated rats. Assessment of mitochondrial indices of functionality revealed a significant decrease in mitochondrial dehydrogenases activity, the collapse of mitochondrial membrane potential, mitochondrial swelling and depletion of ATP content. It was found that boldine supplementation mitigated liver tissue markers of oxidative stress and improved mitochondrial indices of functionality in TAA-treated animals.

Conclusion: The hepatoprotective properties of boldine might primarily rely on antioxidant and mitochondria protecting effects of this alkaloid.

Introduction

Xenobiotics hepatotoxicity is a significant clinical complication. Many xenobiotics including several drugs affect liver function.[1] Hence, finding new therapeutic strategies to protect the liver against toxic insults has clinical value. On the other hand, oxidative stress and its associated complications are among the primary mechanisms underlying xenobiotics-induced hepatotoxicity.[2-4] The thioacetamide-induced liver injury is widely applied as an animal model of hepatic injury.[5] Thioacetamide hepatotoxicity is associated with severe oxidative stress in liver tissue.[5-7] Different cellular targets including biomembrane lipids, proteins, DNA, and cellular mitochondria are affected in thioacetamide model of hepatotoxicity.[6-8]

Boldine is an alkaloid from the boldo tree (*Peumus boldus*). This alkaloid is widely investigated for its antioxidant properties.[9-12] Boldine structurally belongs to the aporphine alkaloids.[13,14] These chemicals are well-known for their antioxidant and radical scavenging activities.[10,13,14] Several pharmacological actions including anti-inflammatory, immunomodulatory, antidiabetic, cytoprotective, and cardiovascular protecting properties have been attributed to boldine.[15-18] Hence, this alkaloid could serve as a potential therapeutic molecule against oxidative stress-mediated injury of biological systems.

This study was designed to evaluate the effect of boldine supplementation and its potential protective mechanisms against liver injury in a rat model. Serum markers of liver injury, tissue histopathological changes, and oxidative stress biomarkers in the liver tissue were monitored. Moreover, several indices of mitochondrial function were assessed to evaluate the potential mechanism of hepatoprotection provided by boldine.

Materials and Methods

Chemicals

Boldine (1,10-Dimethoxy-2,9-dihydroxy aporphine), Thioacetamide, Trichloroacetic acid (TCA), 5,5´–dithionitrobenzoic acid (DTNB), Thiobarbituric acid (TBA), Sodium citrate, Sucrose, KCl, Na_2HPO_4, $MgCl_2$,

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Rhodamine123 (Rh 123), Coomassie brilliant blue, Ethylene glycol-bis (2-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA), Dichlorofluorescein diacetate (DCFDA), and Ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Trichloroacetic acid (TCA), Ferric chloride hexahydrate, 2, 4, 6-Tris (2-pyridyl)-s-triazine (TPTZ), Sodium acetate, Dithiothreitol (DTT), and Hydroxymethyl aminomethane hydrochloride (Tris-HCl) were purchased from Merck (Darmstadt, Germany). Kits for evaluating biomarkers of liver injury, including ALT, LDH, AST, ALP, and bilirubin, were obtained from Pars Azmun® (Tehran, Iran). All salts used for preparing buffer solutions were of analytical grade and obtained from Merck (Darmstadt, Germany).

Animals

Male Sprague-Dawley rats (n = 36; 200-250 g weight) were obtained from the Center for Comparative and Experimental Medicine, Shiraz University of Medical Sciences, Shiraz, Iran. Animals were housed in plastic cages over hardwood bedding. There was an environmental temperature of 24ºC and a 12L: 12D photo schedule along with a ≈40% of relative humidity. Animals were allowed *ad-libitum* access to a regular standard chow diet (Behparvar®, Tehran, Iran) and tap water. Animals received humane care, and all the experiments were performed in conformity with the guidelines for care and use of experimental animals approved by a local ethics committee in Shiraz University of Medical Sciences, Shiraz, Iran (#95-01-36-12118).

Thioacetamide-induced liver injury

Thioacetamide is widely used as a model of acute liver injury.[5] In the current stud, the thioacetamide-induced hepatotoxicity was achieved by a single dose i.p injections of thioacetamide (200 mg/kg).[5] Animals were anesthetized (Thiopental, 50 mg/kg, i.p) and the blood and liver samples were collected 24 hours after thioacetamide administration.[5]

Experimental setup

Rats were allotted in 6 groups (n = 6 in each group) and the treatments were as follows: **1**) Control (Vehicle-treated); **2**) Thioacetamide-treated animals; **3**) Thioacetamide + Boldine (5 mg/kg/day, oral); **4**) Thioacetamide + Boldine (10 mg/kg/day, oral); **5**) Thioacetamide + Boldine (20 mg/kg/day, oral); **6**) Five days of boldine pretreatment (20 mg/kg/day, oral) + Thioacetamide. Boldine alkaloid doses were selected based on previous studies.[19,20] On the other hand, as higher doses of boldine might provide pro-oxidant properties,[21] we preferred to use lower doses of this chemical in the current investigation.

Serum biochemistry and liver histopathology

Standard commercial kits (Pars Azmun®; Tehran, Iran) and a MindrayBS-200® auto analyzer were used to measure serum aspartate aminotransferase (AST), lactate dehydrogenase (LDH), alanine aminotransferase (ALT), bilirubin, and alkaline phosphatase (ALP).[22] For histopathological assessments, samples of liver tissue were fixed in buffered formalin solution (0.64% sodium phosphate dibasic, Na_2HPO_4, 0.4% sodium phosphate monobasic, NaH_2PO_4, and 10% formaldehyde in double distilled water). Afterward, paraffin-embedded sections of tissue (5 µm) were prepared and stained with hematoxylin and eosin (H&E) before light microscope viewing.

Reactive oxygen species (ROS) formation in the liver tissue

Reactive oxygen species in the liver was assessed using DCF-DA as a ROS probe.[23,24] Briefly, liver tissue samples were homogenized in the ice-cooled Tris-HCl buffer (40 mM, 4ºC, pH = 7.4) (1:10 w/v). Aliquots (100 µl) of the resulted tissue homogenate were mixed with Tris-HCl buffer (1 mL) and DCF-DA (Final concentration of 10 µM). The mixture was incubated in the dark (15 minutes, 37ºC). Finally, the fluorescence intensity of the samples was assessed using a FLUOstar Omega® multifunction microplate reader (BMG LABTECH®) ($\lambda_{excitation}$ = 485 nm and $\lambda_{emission}$ = 525 nm).[25]

Lipid peroxidation in liver tissue

The thiobarbituric acid reactive substances (TBARS) test was used as an index of lipid peroxidation in the liver tissue.[26] The reaction mixture consists of 500 µL of tissue homogenate (10% w/v in KCl solution, 1.15% w/v), 3 mL of phosphoric acid (1% w/v, pH = 2) and 1 mL of thiobarbituric acid (0.375%, w/v). Samples were mixed and heated (100 °C, for 45 minutes).[27] After the incubation period, the mixture was cooled, and then 2 mL of n-butanol was added. Samples were vigorously vortexed and centrifuged (10000 *g* for 5 minutes). Finally, the absorbance of developed color in n-butanol phase was read at λ = 532 nm using an Ultrospec 2000®UV spectrophotometer.[26]

Hepatic glutathione reservoirs

Liver samples (200 mg) were homogenized in 8 mL of ice-cooled EDTA (20 mM). Then, 5 ml of the prepared homogenate were added to 4 mL of double distilled water and 1 ml of trichloroacetic acid (TCA; 50% w/v). Samples were mixed well and centrifuged (10,000 *g*, 4°C, 25 minutes). Then, 2 mL of the supernatant was added to 4 mL of Tris-HCl buffer (pH=8.9; 4°C), and 100 µl of DTNB (10 mM in methanol).[28] The absorbance of the developed color was measured at λ = 412 nm using an Ultrospec 2000® UV spectrophotometer.[26]

Total antioxidant capacity of the liver tissue

The ferric reducing antioxidant power (FRAP) of liver tissue was assessed in each experimental group.[29] The working FRAP reagent was prepared by mixing 10 volumes of 300 mmol/L acetate buffer (pH = 3.6), with 1 volume of 10 mmol/L TPTZ (2, 4, 6-tripyridyl-s-triazine, in 40 mmol/L hydrochloric acid) and with 1 volume of 20

mmol/L ferric chloride. All solutions were used on the day of the experiment. Liver tissue was homogenized in 250 mM Tris-HCl buffer containing 200 mM sucrose and 5 mM DTT (pH = 7.4, 4°C). Then, 100 µL of tissue homogenate was added to 1900 µL of the FRAP reagent. The reaction mixture was incubated at 37ºC (5 minutes in the dark). Finally, samples were centrifuged (1000 *g*, 1 min, 4°C) and the absorbance of developed color in the supernatant was measured at λ = 595 nm by an Ultrospec2000® spectrophotometer (Uppsala, Sweden).[30]

Liver mitochondria isolation

Liver tissue mitochondria were isolated using differential centrifugation method.[31] Rat liver was washed and minced in an ice-cold (4°C) buffer medium (70 mM mannitol, 220 mM sucrose, 2 mM HEPES, 0.5 mM EGTA, and 0.1% bovine serum albumin, pH=7.4). The minced tissue was transported into the fresh isolation buffer (10 mL buffer/1g of the kidney tissue, 4°C) and homogenized. First, unbroken cells and nuclei were pelleted (1,000 *g* for 20 min at 4ºC); second; the supernatant was centrifuged (10,000 *g* for 20 min at 4ºC) to pellet the mitochondrial fraction.[31] The recent step was repeated at least three times using fresh buffer medium. Final mitochondrial pellets were suspended in a buffer containing 75 mM Mannitol, 225 mM Sucrose, and 2 mM HEPES, pH=7.4. The mitochondrial preparations used to assess ROS production, mitochondrial depolarization, and mitochondrial swelling, which were suspended in mitochondrial depolarization assay buffer (220 mM Sucrose, 5 mM KH_2PO_4, 68 mM Mannitol, 2 mM $MgCl_2$, 50 µM EGTA, 10 mM KCl, and 10 mM HEPES, pH = 7.2), respiration buffer (125 mM sucrose, 65 mM KCl, 10 mM HEPES, 20 µM Ca^{2+}, sodium succinate 5 mM, pH = 7.2), and swelling buffer (250 mM sucrose, 2 mM HEPES, 0.5 mM KH_2PO_4, 4.2 mM Sodium Succinate; pH = 7.4).[32] Samples' protein concentration was determined by the Bradford method to standardize the obtained data.[33]

Mitochondrial dehydrogenases activity

Based on a previously described procedure, the 3-(4, 5-dimethyl-thiazol-2-yl)-2, the 5-diphenyltetrazolium bromide (MTT) assay was applied as a colorimetric method for determination of mitochondrial dehydrogenases activity.[34,35] Briefly, a mitochondrial suspension (0.5 mg protein/ml) was incubated with 40 µl of 0.4% (w: v) of MTT (37°C for 30 minutes, in the dark). Afterward, samples were centrifuged (16,000 *g*, 10 min) and the pellet (product of purple formazan crystals) was dissolved in 1 mL dimethyl sulfoxide (DMSO). Finally, the optical density (OD) was measured with an EPOCH plate reader (λ = 570 nm, Bio-Tek® Instruments, Highland Park, USA).[36]

Mitochondrial depolarization

A method based on mitochondrial uptake of the cationic fluorescent dye, rhodamine 123, was used to estimate mitochondrial depolarization.[32,37,38] Rhodamine 123, accumulates in intact mitochondria by facilitated diffusion. When the mitochondrion is depolarized and damaged, there is no facilitated diffusion. Hence, the amount of rhodamine 123 in the supernatant is increased.[37,39,40] In the current study, the mitochondrial fractions (0.5 mg protein/ml) were incubated with rhodamine 123 (Final concentration of 10 µM) for 30 minutes.[36] Afterward, samples were centrifuged (16,000 *g*, 10 minutes, 4ºC) and the fluorescence intensity of the supernatant was monitored using a FLUOstar Omega® multi-functional microplate reader ($\lambda_{excitation}$ = 485 nm and $\lambda_{emission}$ = 525 nm).[32,41]

Mitochondrial ATP level

A luciferase-luciferin-based kit (ENLITEN® from Promega) was used to assess mitochondrial ATP content.[42,43] Samples and buffer solutions were prepared based on the kit instructions. Mitochondria samples (1 mg protein/ml) were treated with trichloroacetic acid (TCA; 0.3% w: v in Tris-HCl buffer; 4ºC) and centrifuged (16,000 *g*, 10 min, 4ºC). Then, 100 µl of the supernatant was added to 100 µl of the ATP kit and the luminescence intensity of samples was measured at λ = 560 nm using a FLUOstar Omega® multi-functional microplate reader.[42,43]

Mitochondrial swelling

Changes in the light scattering were used as an estimate of the mitochondrial swelling.[32,41] Briefly, isolated mitochondria were suspended in swelling buffer (250 mM sucrose, 2 mM HEPES, 0.5 mM KH_2PO_4, 4.2 mM Sodium Succinate; pH = 7.4) the mitochondrial permeability transition was initiated by adding calcium (C^{2+}; 200 µM) and assessed by monitoring the absorbance at λ = 540 nm during 30 minutes of incubation using an EPOCH plate reader (Bio-Tek® Instruments, Highland Park, USA). A decrease in the absorbance indicates an increase in mitochondrial swelling.[32,41]

Reactive oxygen species (ROS) in isolated liver mitochondria

The mitochondrial ROS formation was estimated using the fluorescent probe DCFH-DA.[32,44,45] Briefly, isolated liver mitochondria were incubated in a respiratory buffer containing 125 mM sucrose, sodium succinate 5 mM, 65 mM KCl, 10 mM HEPES, 20 µM Ca^{2+}, pH = 7.2 .[32] Following this step, DCFH-DA was added (Final concentration, 10 µM) to mitochondria and then incubated for 30 min at 37ºC. Then, the fluorescence intensity of DCF was measured using a FLUOstar Omega® multifunctional fluorescent microplate reader ($\lambda_{excitation}$ = 485 nm and $\lambda_{emission}$ = 525 nm).[32,45]

Lipid peroxidation in liver mitochondria

Thiobarbituric acid reactive substances (TBARS) were assessed as an index of lipid peroxidation in isolated kidney mitochondria as previously described.[32,41] Briefly, isolated mitochondria were washed to remove sucrose in an ice-cold buffer of MOPS-KCl (50 mM MOPS, 100 mM KCl, pH = 7.4, 4ºC), and re-suspended in fresh

MOPS–KCl buffer. Afterward, the mitochondrial suspension was mixed with twice its volume of 15% trichloroacetic acid, 0.375% thiobarbituric acid (TBA), 0.24 N HCl plus 0.5 mM Trolox, and heated for 15 min at 100ºC.[32,46] After centrifugation (15000 *g*, 10 min), the absorbance of the supernatant was assessed at λ = 532 nm with an Epoch plate reader (BioTek® Instruments, Highland Park, USA).[32,35]

Statistical analysis

Data are given as the Mean±SD. Data comparison was performed by the one-way analysis of variance (ANOVA) with Tukey's multiple comparisons as the *post hoc* test. The differences between groups were considered statistically significant when $P < 0.05$.

Results

Serum biochemical measurement indicated severe liver injury in thioacetamide-treated animals (Figure 1). Significant elevation in serum ALT, AST, bilirubin, and LDH was detected in the TAA-treated group (Figure 1). It was found that boldine supplementation mitigated TAA hepatoxicity as revealed by a significant decrease in the serum liver injury biomarkers (Figure 1). On the other hand, it seems that boldine pre-treatment (20 mg/kg for five consecutive days) had a better effect on serum hepatoxicity biomarkers in comparison with boldine post-treatment (Figure 1).

Liver tissue histopathological changes were evident as severe tissue necrosis, inflammatory cells infiltration, parenchymal hemorrhage, and fatty changes in thioacetamide-treated animals (Figure 2). On the other hand, boldine treatment mitigated thioacetamide-induced histopathological alterations (Figure 2).

Assessment of markers of oxidative stress in the liver tissue of thioacetamide-treated animals revealed a significant increase in reactive oxygen species (ROS) and lipid peroxidation (Figure 3). Moreover, liver tissue glutathione reservoirs were depleted, and tissue antioxidant capacity was significantly decreased in thioacetamide-treated rats (Figure 3). It was found that boldine administration significantly mitigated oxidative stress and its consequences in the liver tissue (Figure 3). On the other hand, boldine pre-treatment had a better effect on liver tissue markers of oxidative stress (Figure 3).

Liver tissue mitochondria were isolated, and several mitochondrial indices were assessed to investigate the possible mechanism of hepatoprotection provided by boldine (Figure 4). It was found that mitochondrial indices of functionality were significantly impaired in thioacetamide model of hepatotoxicity (Figure 4). Mitochondrial dehydrogenases activity was significantly decreased, while mitochondrial ROS formation and lipid peroxidation were increased in the liver mitochondria isolated from thioacetamide-treated animals (Figure 4). On the other hand, significant depletion of mitochondrial ATP content, the collapse of mitochondrial membrane potential, mitochondrial permeabilization, and swelling was also detected in thioacetamide group (Figure 4). It was found that boldine supplementation protected liver mitochondria in thioacetamide-treated animals (Figure 4).

Discussion

Understanding the critical role of oxidative stress and its consequences in the pathogenesis of xenobiotics-induced liver injury have given rise to the search for potent and safe antioxidant molecules. Boldine is a potent aporphine alkaloid.

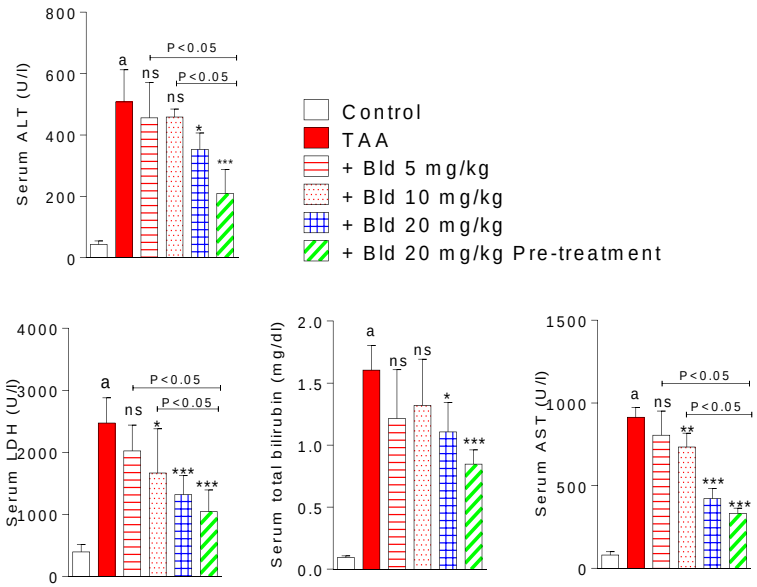

Figure 1. Serum biochemical measurements. Bld: Boldine; TAA: Thioacetamide.
Data are given as Mean±SD (n = 6).
[a] indicate significantly different as compared with control group ($P < 0.001$).
Asterisks indicate significantly different as compared with thioacetamide (TAA) group (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$).
ns: not significant as compared with thioacetamide (TAA) group ($P > 0.05$).

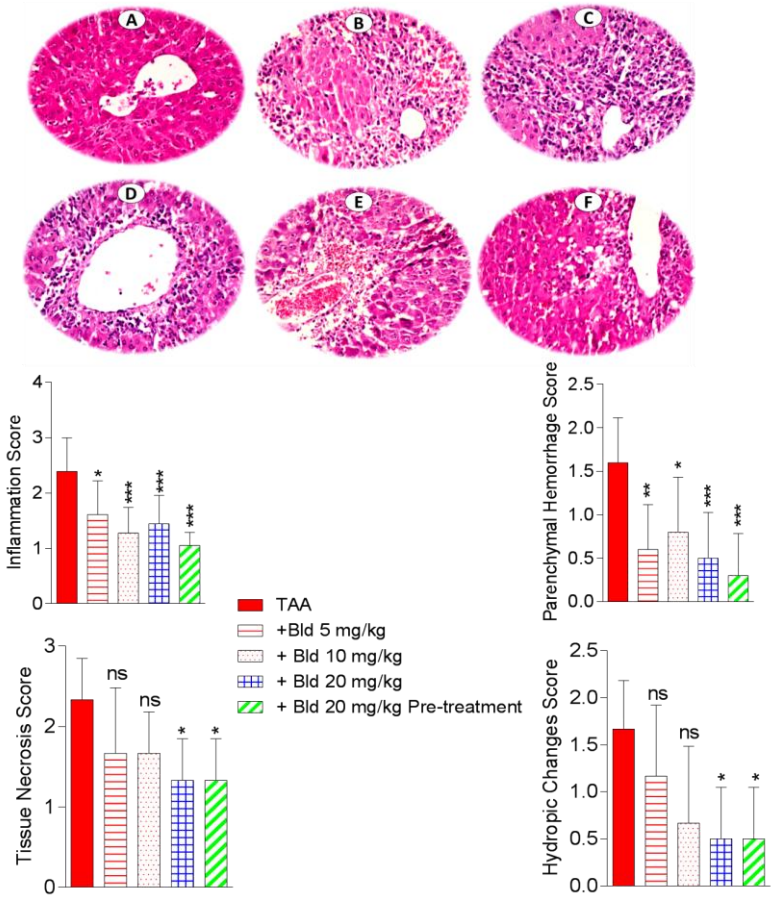

Figure 2. Liver tissue histopathological changes in boldine-treated animals. Bld: Boldine; TAA: Thioacetamide.
Liver photomicrographs represent normal liver tissue (A); thioacetamide-treated group (B); Thioacetamide + Boldine 5, 10, and 20 mg/kg/day/ oral (C, D and E respectively); Boldine pre-treatment (20 mg/kg/day for 5 consecutive days + Thioacetamide (F). Data are given as Mean±SD (n = 6).
Asterisks indicate significantly different as compared with TAA group (*P < 0.05, **P < 0.001, and ***P < 0.001).
ns: not significant as compared with thioacetamide (TAA) group (P > 0.05).

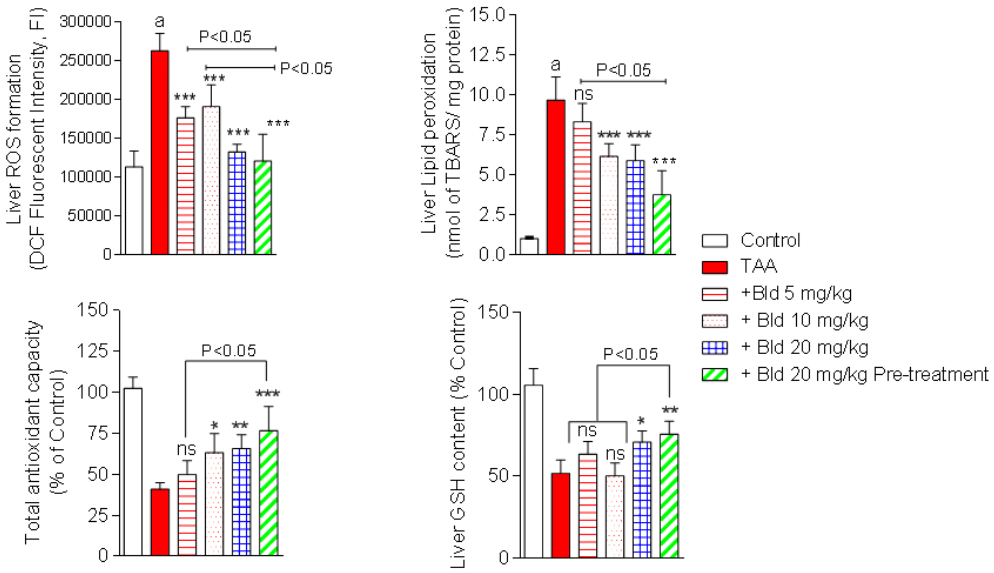

Figure 3. Effect of boldine supplementation on oxidative stress markers in the liver tissue of thioacetamide-treated rats. Bld: Boldine; TAA: Thioacetamide.
Data are represented as Mean ± SD (n = 6).
a Indicates significantly different as compared with control group (P < 0.001).
Asterisks indicate significantly different as compared with TAA group (*P < 0.05; **P<0.01; ***P<0.001).

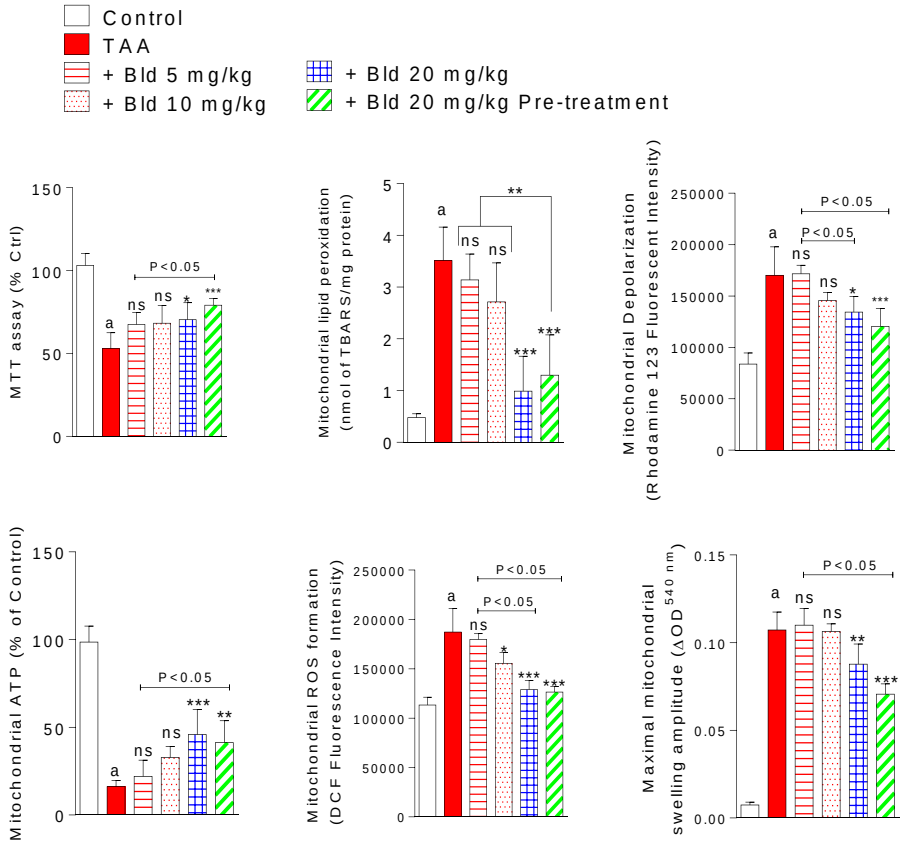

Figure 4. Effect of boldine treatment on liver mitochondrial indices in thioacetamide-treated animals. Bld: Boldine; TAA: Thioacetamide. Data are given as Mean ± SD (n = 6).
[a] Indicates significantly different as compared with control group (P < 0.01).
Asterisks indicate significantly different as compared with TAA group (*P < 0.05, **P < 0.001, and ***P < 0.001).
ns: not significant as compared with thioacetamide (TAA) group (P > 0.05).

Although the protective properties of boldine in several experimental models of human diseases have been tested,[9,47-50] our understanding of the precise mechanism of action of this alkaloid is elusive. The current investigation was designed to evaluate the effect of boldine as a potent antioxidant and its possible mechanism of hepatoprotection in an animal model of liver injury. It was found that boldine treatment (5, 10, and 20 mg/kg, oral) efficiently mitigated thioacetamide-induced hepatotoxicity. This alkaloid also ameliorated markers of oxidative stress, serum biomarkers of liver injury, and liver tissue histopathological lesions. Moreover, boldine efficiently preserved mitochondrial indices of functionality in thioacetamide-intoxicated rats. Oxidative stress and its consequences play an essential role in the pathogeneses of xenobiotics-induced liver injury.[44,51-53] Hence, antioxidant therapy might have therapeutic value. It is well-established that thioacetamide-induced liver failure is associated with severe reactive oxygen/nitrogen species formation, lipid peroxidation, DNA damage, protein carbonylation, and defect in cellular antioxidant defense system.[6-8] Moreover, thioacetamide model of hepatotoxicity could show other aspects of cellular damage including mitochondrial dysfunction.[36]

Aporphine alkaloids are well-known for their antioxidant and radical scavenging properties.[14] On the other hand, boldine has proved the protection of biological targets as an aporphine alkaloid.[12,54] It has been found that boldine efficiently prevented biomembranes lipid oxidation.[12,54,55] The protective effects of boldine against protein modification also have been investigated.[20,54] Boldine also preserved cellular antioxidant capacity at a higher level.[10,20,48] It has also been found that boldine is a very efficient hydroxyl radical ($OH^{\bullet}$) scavenger.[54-56] Hydroxyl radical is the most reactive oxygen species (ROS) which affect different targets or produce other reactive species. The lower rate hepatic lesions could be explained by antioxidant effects of boldine and prevention of deleterious consequences of oxidative stress (*e.g.*, biomembranes disruption) in the current study.[54-56] Hence, an essential mechanism for the hepatoprotective properties of boldine could be mediated through its antioxidant capacity.

Cellular mitochondria are involved in many physiological processes including the cell death.[57,58] The mechanisms of xenobiotics-induced liver injury sometimes rely on mitochondrial dysfunction.[35,59,60] On the other hand, oxidative stress and mitochondrial injury are mechanistically interconnected.[61] It has been shown that mitochondrial dysfunction occurs in animal models of thioacetamide hepatotoxicity.[36] In the current study,

severe decrease in mitochondrial function was detected. It was found that mitochondrial membrane potential was collapsed and mitochondrial ATP content was decreased in thioacetamide-treated rats. Moreover, a significant decrease in mitochondrial dehydrogenases activity and increased mitochondrial swelling and ROS formation was detected (Figure 4). These data might indicate that hepatocytes mitochondrial dysfunction could play a significant role in the mechanism of liver injury induced by thioacetamide. On the other hand, we found that boldine supplementation preserved mitochondrial function in thioacetamide-treated animals.

The effect of boldine on cellular mitochondria has been mentioned in previous investigations.[47,62] It has been found that boldine inhibited nitric oxide production by mitochondria.[63] In the current study, we found that boldine treatment prevented thioacetamide-induced deterioration of mitochondrial function (Figure 4). It was also found that mitochondria-originated ROS was also lower in boldine-treated groups (Figure 4). These data indicate that regulating mitochondrial function could serve as an essential mechanism for the hepatoprotective properties of boldine. On the other hand, oxidative stress might hasten mitochondrial dysfunction and *vice versa*.[61] Hence, the mechanism of mitochondria protecting effects of boldine observed in the current investigation could also be attributed, at least in part, to the antioxidant properties of this alkaloid.

The anti-inflammatory effects of boldine have been mentioned in several studies.[17,62,64,65] It has been shown that this alkaloid ameliorated inflammatory cells infiltration as well as prostaglandins and cytokines release.[17,64] Hepatic inflammation plays an essential role in the pathogenesis of liver injury in the thioacetamide model,[66] or other types of xenobiotics-induced hepatotoxicity.[27,67] In the current study we found that inflammatory cells infiltration was significantly lower in boldine-treated groups (Figure 2). Hence, the effect of boldine treatment on hepatic inflammation and inflammatory mediators could be the subject of further research in this field.

Increased oxidative stress and its associated complications have been suggested to be involved in several diseases.[68,69] Some studies mentioned the importance of boldine in the treatment of oxidative stress-associated pathological conditions in human.[14] This mention the potential therapeutic use of safe and potent antioxidants (*e.g.*, boldine) against these complications.

Conclusion

The data obtained from the current study suggest that regulation of mitochondrial function along with antioxidant and radical scavenging properties of boldine is a primary mechanism underlying the hepatoprotective properties of this chemical. Further studies might reveal the clinical significance of these data as well as the potential application of this potent antioxidant alkaloid against several other oxidative stress-mediated disorders.

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Conflict of interests

The authors claim that there is no conflict of interest.

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