Original Article

Hepatoprotective Effects of Betaine Against Isoniazid-Induced Liver Injury in Rats: Biochemical, Oxidative Stress, and Histopathological Evidence

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Received Date: 25 June 2026; **Revised Date:** 23 August2026; **Accepted Date**: 26 August 2026; **Published Date:** 30 August 2026.

Nexus of Pathophysiology and Therapeutics (NPT). 2026;1(1): 015, https://doi.org/10.22034/npt.2026.1.015

Abstract:

Background: Isoniazid (INH) is a first-line antitubercular drug; however, its clinical use may be complicated by drug-induced liver injury, associated with oxidative stress and disruption of hepatic homeostasis. Betaine is a naturally occurring methyl donor with reported antioxidant and hepatoprotective properties. This study evaluated the protective effects of different betaine doses against INH-induced hepatic injury in rats.

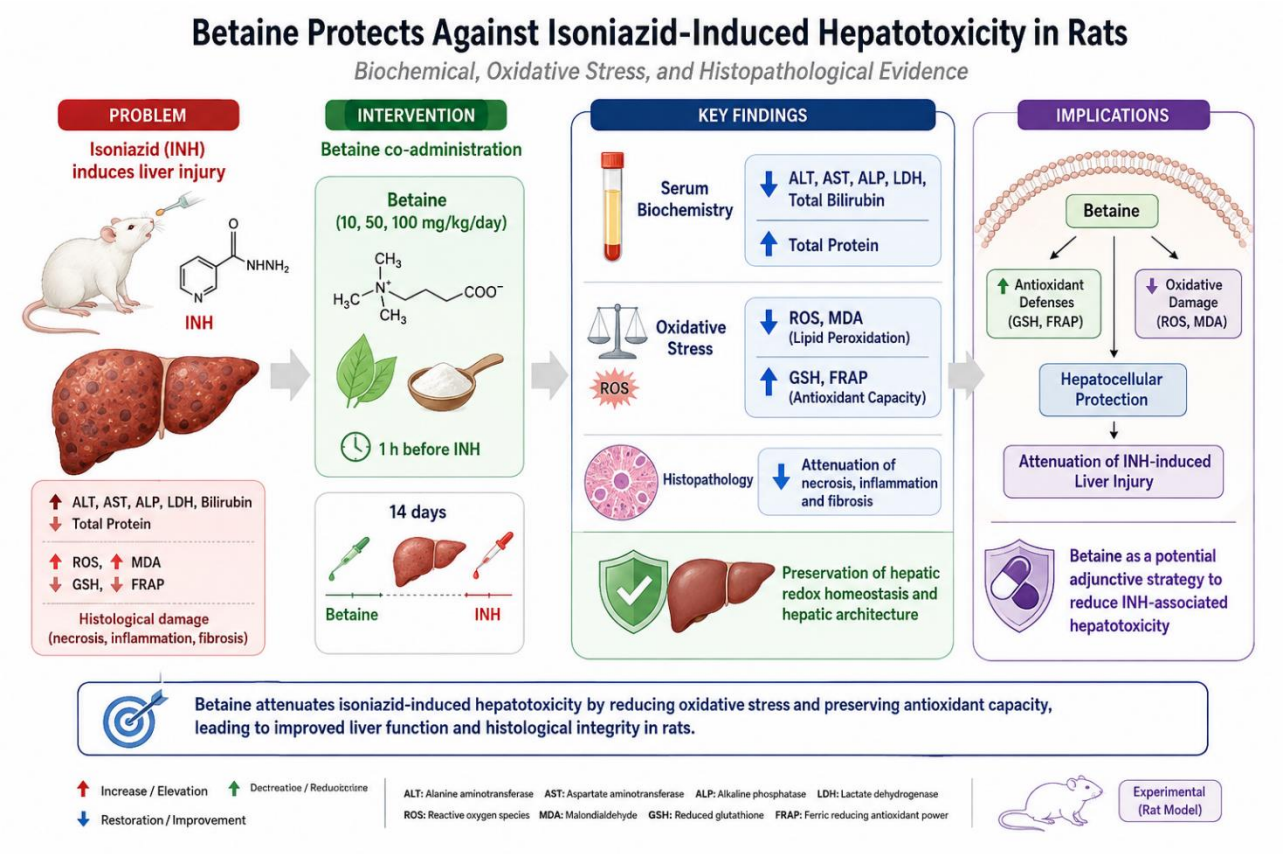
Methods: Sixty adult male Wistar rats were randomly divided into six experimental groups, with 10 animals per group: control, INH (300 mg/kg/day), INH plus betaine (10, 50, or 100 mg/kg/day), and betaine alone (100 mg/kg/day). Treatments were administered orally for 14 consecutive days, with betaine given 1 h before INH. Serum biochemical markers, including alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, lactate dehydrogenase, total bilirubin, and total protein, were evaluated. Hepatic glutathione (GSH), malondialdehyde (MDA), reactive oxygen species (ROS), and ferric reducing antioxidant power (FRAP) were measured. Liver alterations were assessed by hematoxylin and eosin (H&E) staining.

Results: INH administration significantly elevated serum ALT, AST, ALP, LDH, and total bilirubin levels, while reducing total protein, GSH, and FRAP and increasing hepatic ROS and MDA levels. Betaine at 10, 50, and 100 mg/kg significantly attenuated these biochemical and oxidative alterations and improved hepatic histopathology, without significant effects when administered alone.

Conclusion: Betaine attenuated INH-induced biochemical, oxidative, and histopathological alterations in rat liver, suggesting that preservation of hepatic redox homeostasis may contribute to its hepatoprotective effects. These findings support further preclinical investigation of betaine as a potential adjunctive strategy for limiting INH-associated hepatotoxicity.

Mechanistic and Translational Relevance: The hepatoprotective effects of betaine were accompanied by restoration of hepatic redox homeostasis, characterized by increased GSH and FRAP and reduced ROS and MDA levels. These findings provide a mechanistically informed basis for further preclinical investigation of betaine as a potential adjunctive strategy for attenuating INH-associated hepatotoxicity.

Keywords: Isoniazid; Betaine; Hepatotoxicity; Oxidative Stress; Lipid Peroxidation; Drug-Induced Liver Injury



Graphical Abstract: Betaine attenuates isoniazid-induced hepatotoxicity by preserving hepatic redox homeostasis and reducing liver injury. Isoniazid exposure induced hepatic injury characterized by increased serum liver injury markers, oxidative stress, lipid peroxidation, depletion of glutathione, and impaired antioxidant capacity, accompanied by histopathological damage. Betaine co-administration attenuated these biochemical and oxidative alterations, increased hepatic GSH and FRAP, reduced ROS and MDA levels, and improved hepatic histopathological features. These findings support further preclinical investigation of betaine as a potential adjunctive strategy for limiting isoniazid-associated hepatotoxicity.

Introduction

Isoniazid (INH) remains a cornerstone of antitubercular therapy worldwide and is widely used as a first-line agent in the treatment of tuberculosis (1). Despite its clinical efficacy, the use of INH is limited by drug-induced liver injury (DILI), which represents one of its most clinically important adverse effects (2). The pathogenesis of INH-induced hepatotoxicity is multifactorial and has been associated with the formation of reactive metabolites, oxidative stress, mitochondrial dysfunction, and disruption of hepatic redox homeostasis (3). These alterations may promote lipid peroxidation, hepatocellular injury, and inflammatory responses, ultimately impairing hepatic function (4). Therefore, effective strategies to prevent or attenuate INH-induced hepatotoxicity remain clinically relevant. Betaine (trimethylglycine) is a naturally occurring methyl donor that participates in one-carbon metabolism and contributes to the maintenance of cellular osmotic and metabolic homeostasis (5). By facilitating the remethylation of homocysteine to methionine, betaine supports methylation reactions

and cellular metabolic processes. In addition, its function as an organic osmolyte may contribute to the preservation of cellular integrity under conditions of metabolic and oxidative stress (6). Previous experimental studies have reported hepatoprotective effects of betaine in different models of liver injury, including effects associated with attenuation of oxidative stress and preservation of mitochondrial function (7). These properties suggest that betaine may influence pathways involved in the development of drug-induced liver injury. Oxidative stress is considered an important contributor to INH-induced hepatotoxicity. Excessive generation of reactive oxygen species (ROS) may contribute to glutathione (GSH) depletion, impairment of antioxidant defenses, and increased lipid peroxidation, thereby promoting hepatocellular injury (8, 9). Hepatic injury may also disrupt metabolic processes involved in ammonia clearance and thereby alter hepatic ammonia homeostasis (10). Given its reported effects on redox balance and cellular metabolism, betaine may represent a potential protective agent against the oxidative and metabolic disturbances associated with INH-induced liver injury (7). However, the hepatoprotective effects of betaine and their association with oxidative and metabolic alterations in INH-induced hepatotoxicity remain insufficiently characterized. Therefore, the present study aimed to evaluate the protective effects of different doses of betaine in a rat model of INH-induced liver injury. Hepatic injury was assessed using serum biochemical markers, histopathological examination, and hepatic oxidative stress-related parameters, including reactive oxygen species (ROS), reduced glutathione (GSH), lipid peroxidation, and ferric reducing antioxidant power (FRAP). Hepatic ammonia levels were also evaluated to explore potential alterations in hepatic metabolic function. By integrating biochemical, oxidative, metabolic, and histopathological outcomes, this study aimed to characterize the hepatoprotective effects of betaine against INH-induced hepatic injury and to provide further insight into its association with oxidative and metabolic alterations.

Materials and Methods

Chemicals and Reagents

Throughout the experimental procedures, all reagents and chemical compounds were obtained in analytical-grade purity and used without further modification. Isoniazid (INH), betaine, thiobarbituric acid (TBA), potassium chloride (KCl), metaphosphoric acid, 2′,7′-dichlorofluorescin diacetate (DCFH-DA), 2,4,6-tripyridyl-s-triazine (TPTZ), 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB), trichloroacetic acid (TCA), and ethylenediaminetetraacetic acid (EDTA) were used as received. Fresh working solutions of the required reagents were prepared immediately before each assay according to the respective assay protocols.

Experimental Animals

Sixty adult male Wistar rats weighing 180–220 g were used in this study. The animals were acclimatized for 1 week under standard laboratory conditions at 22 ± 2°C with a 12-h light/12-h dark cycle and had free access to standard laboratory chow and water throughout the acclimatization and experimental periods. Following acclimatization, the rats were randomly allocated to six experimental groups (n = 10 per group). All animal experiments were performed in compliance with the Guide for the Care and Use of Laboratory Animals (11), as well as relevant institutional and national regulations governing the care and use of laboratory animals. The study was approved by the Institutional Animal Ethics Committee of Lorestan University of Medical Sciences (Approval No. IR.LUMS.REC.1401.036). Measures were taken

throughout the study to minimize animal discomfort and distress while limiting the number of animals required for the experiments.

Experimental Design and Treatment Groups

The animals were randomly distributed among six experimental groups, with 10 rats in each group. Rats in the control group were administered normal saline by oral gavage once daily for 14 consecutive days. Animals in the INH group were administered isoniazid at a dose of 300 mg/kg/day by oral gavage for 14 consecutive days (12). Three treatment groups received betaine at doses of 10, 50, or 100 mg/kg/day by oral gavage 1 h before the daily administration of INH for 14 consecutive days (12). A separate betaine-only group received betaine at 100 mg/kg/day by oral gavage for 14 consecutive days to assess the effects of the highest betaine dose in the absence of INH. Following completion of the treatment period, anesthesia was induced with sodium pentobarbital (60 mg/kg, i.p.). Adequate anesthetic depth was verified before blood and tissue samples were collected.

Blood Collection and Serum/Plasma Preparation

Upon completion of the experimental protocol, blood samples were obtained from the abdominal aorta while the animals remained under deep anesthesia. Approximately 5 mL of blood was obtained from each rat. For serum preparation, 3 mL of blood was transferred into plain tubes and allowed to clot at room temperature. The samples were then centrifuged at 1,000 × g for 25 min, and the separated serum was collected and stored at −20°C until biochemical analysis. The remaining 2 mL of blood was collected into EDTA-containing tubes for plasma preparation. Plasma was obtained by centrifuging the blood samples at 1,000 × g for 10 min at 4°C and was subsequently used to quantify ammonia levels. Samples intended for ammonia analysis were processed promptly to minimize changes in plasma ammonia concentrations during sample handling.

Biochemical Assays

Serum biochemical analysis was performed to assess markers of hepatic injury, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), bilirubin, and total protein (13). Serum biochemical parameters were determined using commercially available diagnostic kits (Pars Azmoon Co., Tehran, Iran) according to the manufacturers' instructions.

Plasma ammonia concentrations were determined using a colorimetric indophenol method, as previously described (14). Briefly, 100 µL of plasma was mixed with 720 µL of deionized water, 40 µL of phenol, 40 µL of sodium nitroprusside, and 100 µL of an oxidizing reagent. The reaction mixture was incubated in the dark at room temperature for 60 min, and the absorbance of the resulting indophenol blue complex was measured at 625 nm using a microplate reader. Each measurement was conducted in triplicate, and ammonia concentrations were determined using a standard curve generated from known ammonium chloride concentrations.

Tissue Collection and Preparation

Following blood collection, the liver was rapidly excised and rinsed thoroughly with ice-cold 0.9% physiological saline to remove residual blood. Liver tissue specimens were collected and separately prepared for subsequent biochemical and oxidative stress assessments. Tissue samples were weighed and homogenized in ice-cold assay-specific buffers as described below. The remaining liver tissue was fixed in 10% neutral buffered formalin for subsequent histopathological examination.

Hepatic Biochemical and Oxidative Stress Assessments

Liver tissue samples were homogenized in ice-cold buffers appropriate for each assay. Total protein content was determined using the Bradford method and was used for normalization of biochemical measurements where applicable. Standard calibration curves were generated for quantitative assays, including malondialdehyde (MDA), reduced glutathione (GSH), and ferric reducing antioxidant power (FRAP). The corresponding calibration curves are provided in the Supplementary Material.

Lipid Peroxidation (TBARS)

Ice-cold 1.15% KCl-prepared liver homogenates were treated with 1% phosphoric acid and 0.6% thiobarbituric acid (TBA), followed by incubation at 100°C for 45 min. The reaction products were extracted with n-butanol, and the absorbance of the organic phase was measured at 532 nm. MDA concentration was calculated using a standard curve prepared with malondialdehyde and expressed relative to tissue weight.

Reduced Glutathione (GSH)

Liver tissue homogenization was performed in 0.02 M EDTA at a 1:10 (w/v) ratio, followed by deproteinization with 50% trichloroacetic acid. The homogenates were centrifuged at 3,000 × g for 15 min. The supernatant was subsequently combined with 0.4 M Tris buffer (pH 8.9) and 0.01 M 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) for the reaction. Absorbance was measured at 412 nm within 5 min. GSH concentrations were calculated from a standard curve prepared using reduced glutathione and expressed as nmol/g tissue.

Ferric Reducing Antioxidant Power (FRAP)

A 100-µL aliquot of the liver homogenate was added to freshly prepared FRAP reagent composed of 300 mM acetate buffer (pH 3.0), 20 mM $FeCl_3$, and 1 mM 2,4,6-tripyridyl-s-triazine (TPTZ). The mixture was allowed to stand at room temperature for 5 min, after which absorbance was recorded at 593 nm with a microplate reader. FRAP values were calculated from a standard calibration curve and expressed as µmol ascorbic acid equivalents per gram of liver tissue.

Reactive Oxygen Species (ROS)

Liver homogenates prepared in ice-cold Tris-HCl buffer (40 mM, pH 7.4) were incubated with 1 µM 2′,7′-dichlorofluorescin diacetate (DCFH-DA) for 15 min at 37°C in the dark. Fluorescence was recorded using excitation and emission wavelengths of approximately 485 and 520 nm, respectively (13).

Histopathological Examination

Following tissue collection for biochemical and oxidative stress analyses, representative liver specimens were rinsed with 0.9% physiological saline and fixed in 10% neutral buffered formalin for 24 h. The tissue specimens were dehydrated using graded ethanol, cleared with xylene, and embedded in paraffin. Following paraffin embedding, tissue sections (5 µm thick) were prepared with a rotary microtome, mounted on glass slides, and subjected to hematoxylin and eosin (H&E) staining. The stained specimens were subsequently evaluated under light microscopy for histopathological changes (13).

Statistical Analysis

Data were analyzed using GraphPad Prism version 6.0 (GraphPad Software, San Diego, CA, USA) and are presented as mean ± standard error of the mean (SEM). Comparisons between experimental groups were performed by one-way analysis of variance (ANOVA), with Tukey's multiple comparisons test applied for post hoc analysis. Statistical significance was defined as a two-sided P value < 0.05.

Results

Effect of Betaine on Serum Total Bilirubin Levels

Compared with the control group, rats treated with isoniazid (INH) exhibited a significant elevation in serum total bilirubin levels (***P < 0.001). Co-administration of betaine at 10, 50, and 100 mg/kg significantly attenuated the INH-induced increase in serum bilirubin levels compared with the INH group (^aP < 0.05; Figure 1). Administration of betaine alone at 100 mg/kg did not significantly alter serum bilirubin levels compared with the control group.

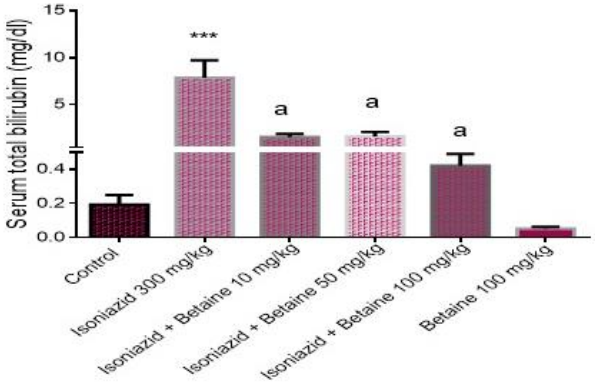


Figure 1. Effect of betaine on serum total bilirubin levels in isoniazid-treated rats. Serum total bilirubin levels were measured following isoniazid administration with or without betaine treatment at 10, 50, and 100 mg/kg. Data are presented as mean ± SEM. ***P < 0.001 vs. control; ^aP < 0.05 vs. INH group.

Effect of Betaine on Serum Alkaline Phosphatase Levels

Serum alkaline phosphatase (ALP) levels were significantly increased in the isoniazid (INH)-treated group compared with the control group (***P < 0.001; Figure 2). Treatment with betaine (10, 50, and 100 mg/kg) significantly reduced the INH-associated increase in serum ALP levels relative to the INH group (^aP < 0.05). Administration of betaine alone at 100 mg/kg did not significantly alter serum ALP levels compared with the control group.

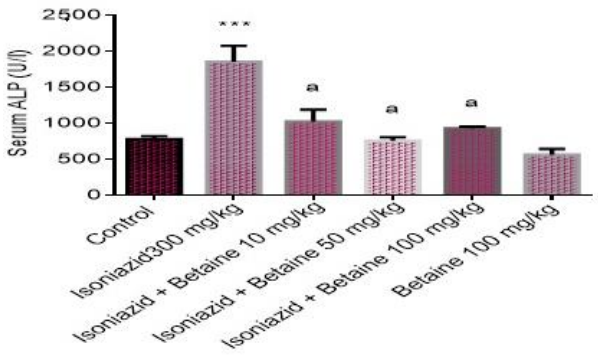


Figure 2. Effect of betaine on serum alkaline phosphatase (ALP) levels in isoniazid-treated rats. Serum ALP levels were measured following isoniazid administration with or without betaine treatment at 10, 50, and 100 mg/kg. Data are presented as mean ± SEM. ***P < 0.001 vs. control; ^aP < 0.05 vs. INH group.

Effect of Betaine on Serum Lactate Dehydrogenase Levels

A significant elevation in serum LDH levels was observed following INH administration compared with the control group (***P < 0.001; Figure 3). Co-administration of betaine at 10, 50, and 100 mg/kg significantly attenuated the INH-induced increase in serum LDH levels compared with the INH group (^aP < 0.05). Administration of betaine alone at 100 mg/kg did not significantly alter serum LDH levels compared with the control group.

Effect of Betaine on Serum Alanine Aminotransferase Levels

A significant increase in serum alanine aminotransferase (ALT) levels was observed in rats receiving isoniazid (INH) compared with the control group (***P < 0.001; Figure 4). Co-administration of betaine at 10, 50, and 100 mg/kg significantly attenuated the INH-induced increase in serum ALT levels compared with the INH group (^aP < 0.05). Administration of betaine alone at 100 mg/kg did not significantly alter serum ALT levels compared with the control group.

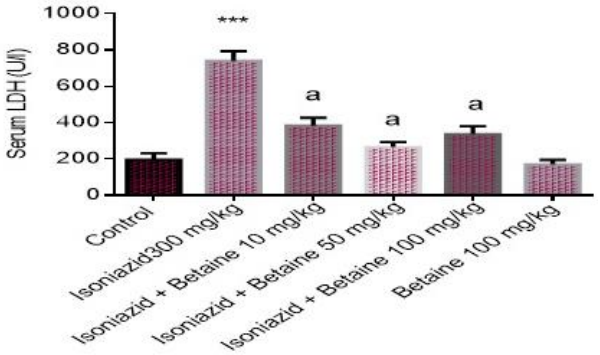


Figure 3. Effect of betaine on serum lactate dehydrogenase (LDH) levels in isoniazid-treated rats. Serum LDH levels were measured following isoniazid administration with or without betaine treatment at 10, 50, and 100 mg/kg. Data are presented as mean ± SEM. ***P < 0.001 vs. control; ^aP < 0.05 vs. INH group.

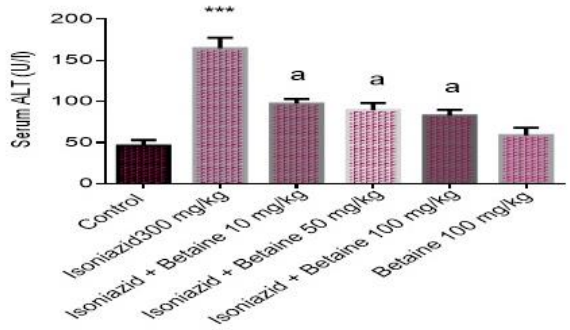


Figure 4. Effect of betaine on serum alanine aminotransferase (ALT) levels in isoniazid-treated rats. Serum ALT levels were measured following isoniazid administration with or without betaine treatment at 10, 50, and 100 mg/kg. Data are presented as mean ± SEM. ***P < 0.001 vs. control; ^aP < 0.05 vs. INH group.

Effect of Betaine on Serum Aspartate Aminotransferase Levels

Rats exposed to INH showed a significant elevation in serum AST levels relative to the control group (***P < 0.001; Figure 5). Betaine treatment at 10, 50, and 100 mg/kg significantly reduced the INH-associated increase in serum AST levels compared with the INH group (^aP < 0.05). In contrast, betaine administered alone at 100 mg/kg produced no significant change in serum AST levels relative to the control group.

Effect of Betaine on Serum Total Protein Levels

A significant reduction in serum total protein levels was observed in the INH-treated rats compared with the control group (^aP < 0.05; Figure 6). Betaine treatment mitigated the INH-related decrease in serum total protein at all tested doses (10, 50, and 100 mg/kg), reaching statistical significance at P < 0.05 for 10 mg/kg and P < 0.01 for both 50 and 100 mg/kg. Administration of betaine alone at 100 mg/kg did not significantly alter serum total protein levels compared with the control group.

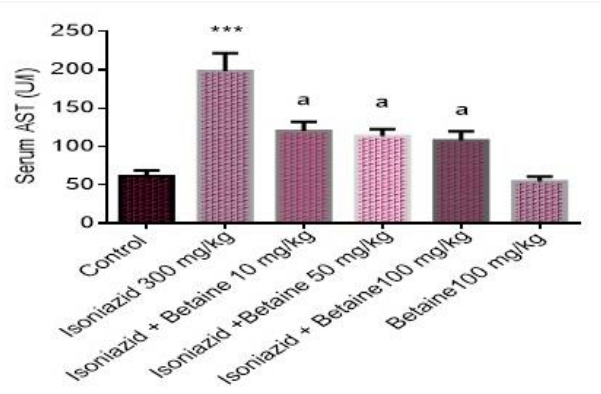


Figure 5. Effect of betaine on serum aspartate aminotransferase (AST) levels in isoniazid-treated rats. Serum AST levels were measured following isoniazid administration with or without betaine treatment at 10, 50, and 100 mg/kg. Data are presented as mean ± SEM. ***P < 0.001 vs. control; ^aP < 0.05 vs. INH group.

Effects of Betaine on Hepatic Glutathione and Lipid Peroxidation Levels

Hepatic biochemical analysis showed that INH administration significantly decreased reduced GSH levels and increased lipid peroxidation, as indicated by elevated MDA levels, compared with the control group (*P < 0.05; Table 1). Co-administration of betaine significantly attenuated these oxidative alterations, with increased GSH levels and reduced MDA levels compared with the INH group (^bP < 0.05; Table 1). The betaine-only group showed GSH and MDA levels comparable to those of the control group.

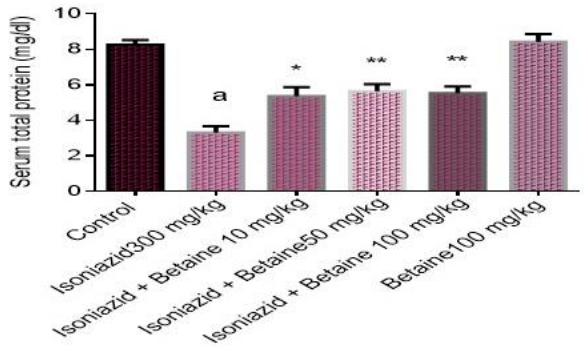


Figure 6. Effect of betaine on serum total protein levels in isoniazid-treated rats. Serum total protein levels were measured in control, isoniazid (INH)-treated, INH + betaine (10, 50, and 100 mg/kg), and betaine (100 mg/kg)-treated rats. Data are presented as mean ± SEM. ^aP < 0.05 vs. control; *P < 0.05 and **P < 0.01 vs. INH group.

Groups	Liver GSH content (µmol/mg)	Liver TBARS (nmol/mg wet tissue)
Control	61.31 ± 6.14	1.33 ± 0.26
Betaine 100 mg/kg	74.38 ± 1.12	2.03 ± 0.08
Isoniazid	19.71 ± 5.18*	11.61 ± 1.52*
Isoniazid + Betaine 10 mg/kg	39.74 ± 3.53#	5.19 ± 0.16#
Isoniazid + Betaine 50 mg/kg	52.22 ± 2.61#	3.92 ± 0.71#
Isoniazid + Betaine 100 mg/kg	50.44 ± 7.04#	2.11 ± 0.62#

Table 1. Effects of betaine on hepatic reduced glutathione (GSH) and malondialdehyde (MDA) levels in isoniazid-induced hepatotoxicity. Data are presented as mean ± SEM (n = 10 per group). ^aP < 0.05 vs. the control group; ^bP < 0.05 vs. the INH group.

Effect of Betaine on Hepatic Reactive Oxygen Species Levels

INH exposure resulted in a significant elevation of hepatic ROS levels relative to the control group (***P < 0.001; Figure 7). Treatment with betaine at 10, 50, and 100 mg/kg significantly reduced hepatic ROS levels compared with the INH group (^aP < 0.05). Administration of betaine alone at 100 mg/kg did not significantly alter hepatic ROS levels compared with the control group.

Effect of betaine on hepatic FRAP levels

INH administration significantly reduced hepatic FRAP values relative to the control group (***P < 0.001; Figure 8). Conversely, co-treatment with betaine at doses of 10, 50, and 100 mg/kg significantly restored hepatic FRAP levels compared with those observed in the INH-treated group (aP < 0.05). Administration of betaine alone at 100 mg/kg did not significantly alter hepatic FRAP values compared with the control group.

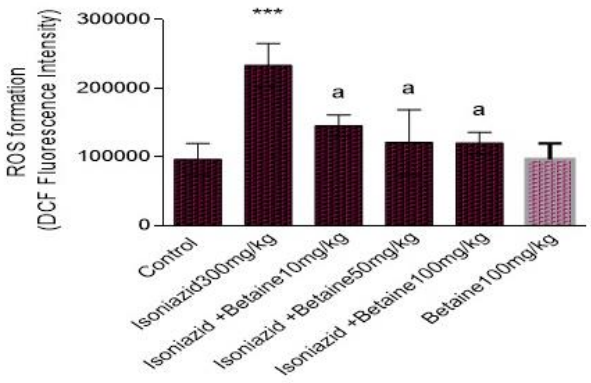


Figure 7. Effect of betaine on hepatic reactive oxygen species (ROS) levels in isoniazid-treated rats. Hepatic ROS levels were measured following isoniazid administration with or without betaine treatment at 10, 50, and 100 mg/kg. Data are presented as mean ± SEM. ***P < 0.001 vs. control; ^aP < 0.05 vs. INH group.

Histopathological Evaluation of Hepatic Tissue

Histopathological alterations in liver tissue, evaluated by hematoxylin and eosin (H&E) staining, are summarized in Table 2. The INH-treated group exhibited marked hepatic histopathological alterations, including moderate focal necrosis (++), severe portal inflammation (+++), and moderate bile duct proliferation (++), with an Ishak fibrosis stage of 4. Co-administration of betaine attenuated several of these histopathological alterations. The 10 mg/kg betaine group showed mild focal necrosis (+) and mild portal inflammation (+), with no bile duct proliferation and an Ishak fibrosis stage of 3.

In the 50 and 100 mg/kg betaine groups, focal necrosis was absent, while portal inflammation and bile duct proliferation remained mild (+), with an Ishak fibrosis stage of 2.

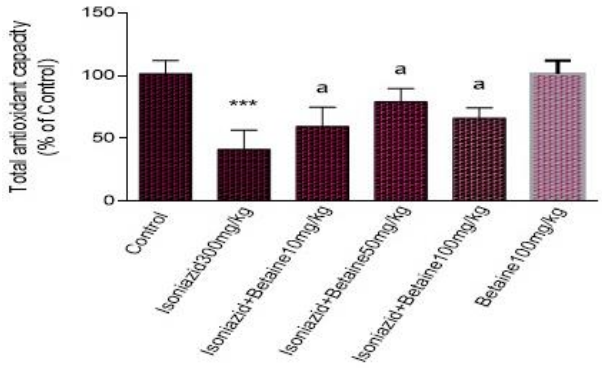


Figure 8. Effect of betaine on hepatic ferric reducing antioxidant power (FRAP) levels in isoniazid-treated rats. Hepatic FRAP levels were measured following isoniazid administration with or without betaine treatment at 10, 50, and 100 mg/kg. Data are presented as mean ± SEM. ***P < 0.001 vs. control; ^aP < 0.05 vs. INH group.

Discussion

The present study investigated the protective effects of betaine against isoniazid (INH)-induced hepatic injury in rats, with particular emphasis on serum biochemical markers, hepatic oxidative stress, and histopathological alterations. The principal findings demonstrated that INH administration resulted in significant increases in serum ALT, AST, ALP, LDH, and total bilirubin levels, accompanied by a reduction in serum total protein. These biochemical alterations were associated with increased hepatic ROS and MDA levels and decreased GSH and FRAP, indicating a substantial disruption of hepatic redox homeostasis. Histopathological examination further demonstrated structural alterations following INH exposure. Co-administration of betaine attenuated these biochemical, oxidative, and histopathological changes, supporting its hepatoprotective potential against INH-induced liver injury.

Treatment group	Confluent necrosis	Focal necrosis	Portal inflammation	Bile duct proliferation	Ishak fibrosis
Control	–	–	–	–	0
INH	–	++	+++	++	4
INH + Betaine 10 mg/kg/day	–	+	+	–	3
INH + Betaine 50 mg/kg/day	–	–	+	+	2
INH + Betaine 100 mg/kg/day	–	–	+	+	2

Table 2. Histopathological effects of betaine on liver injury in isoniazid-induced hepatotoxicity. Liver sections were stained with hematoxylin and eosin (H&E) and evaluated for histopathological alterations, including confluent necrosis, focal necrosis, portal inflammation, and bile duct proliferation. The severity of histopathological lesions was graded semiquantitatively as follows: –, absent; +, mild; ++, moderate; +++, severe. Hepatic fibrosis was assessed according to the Ishak fibrosis scoring system and scored from 0 to 6.

Isoniazid remains an important first-line antitubercular drug; however, its use can be complicated by drug-induced liver injury (DILI) (15). The mechanisms underlying INH-induced hepatotoxicity are multifactorial and have been associated with reactive metabolites, oxidative stress, mitochondrial dysfunction, and inflammatory responses (3, 4, 10, 16). In the present study, the elevation of serum ALT, AST, ALP, LDH, and bilirubin following INH administration is consistent with hepatic cellular injury and impaired hepatic function. These findings are in agreement with previous experimental and clinical observations describing biochemical and structural hepatic alterations following INH exposure (2, 12, 16).

A prominent finding of the present study was the marked alteration of hepatic oxidative stress parameters following INH administration. INH exposure resulted in decreased hepatic GSH and

FRAP values, together with increased ROS and MDA levels. GSH represents an important component of the endogenous hepatic antioxidant defense system, whereas increased MDA reflects enhanced lipid peroxidation. The simultaneous depletion of antioxidant defenses and elevation of oxidative damage markers suggests that INH disrupted hepatic redox homeostasis. Previous studies have similarly implicated oxidative stress and mitochondrial dysfunction in INH-induced hepatic injury (3, 4, 9, 17). Betaine administration significantly attenuated these oxidative alterations, as demonstrated by increased hepatic GSH and FRAP values and decreased ROS and MDA levels. These findings are consistent with previous experimental studies reporting antioxidant and hepatoprotective effects of betaine in different models of hepatic injury (18, 19). Betaine has also been reported to influence mitochondrial respiration and cellular metabolic homeostasis, providing a potential basis for its protective effects under conditions of hepatic oxidative stress (20-22). Nevertheless, the present study did not directly assess mitochondrial respiratory function or specific molecular signaling pathways; therefore, these mechanisms should be considered potential explanations rather than demonstrated mechanisms in the current model. The protective effects of betaine were also reflected in the serum biochemical findings. Co-administration of betaine reduced the INH-induced elevations in ALT, AST, ALP, LDH, and bilirubin and attenuated the reduction in serum total protein. Similar hepatoprotective effects of betaine have been reported in other experimental models of drug- or toxin-induced hepatic injury, including rifampicin-induced liver injury and acetaminophen-induced hepatotoxicity (23, 24). Experimental evidence has also suggested that betaine may protect hepatic tissue against oxidative and metabolic stress through effects on cellular redox regulation and mitochondrial function (7, 18, 19). The histopathological findings provided additional evidence supporting the biochemical results. INH administration was associated with focal necrosis, portal inflammation, and bile duct proliferation, together with an Ishak fibrosis stage of 4. Co-administration of betaine attenuated several of these structural alterations. In particular, focal necrosis was absent in the 50 and 100 mg/kg betaine groups, while the Ishak fibrosis stage was reduced to 2 in both groups. These findings are consistent with previous reports demonstrating that oxidative stress and disruption of antioxidant defenses contribute to structural hepatic injury during toxicant exposure (17, 25). The mechanisms underlying the hepatoprotective effects of betaine are likely to be multifactorial. In addition to its role as a methyl donor and organic osmolyte, betaine has been reported to influence cellular membrane integrity, mitochondrial function, antioxidant defenses, and inflammatory signaling (5, 21, 26). Experimental studies have shown that betaine can protect membrane lipid composition and membrane-associated ATPase activity under conditions of ethanol-induced injury (27). Moreover, betaine has been reported to exert anti-inflammatory effects in experimental models of tissue injury (28-30). These properties may collectively contribute to the attenuation of INH-induced hepatic damage observed in the present study. The antioxidant effects of betaine may not necessarily result from direct free-radical scavenging. Previous evidence suggests that betaine can modulate endogenous antioxidant mechanisms and improve cellular redox homeostasis without acting as a conventional free-radical scavenger (31).

In addition, activation of the Nrf2/HO-1 pathway has been proposed as a potential mechanism of betaine-mediated cytoprotection in other experimental models (32). However, because the present study did not measure Nrf2, HO-1, Keap1, or other related molecular markers, the involvement of these pathways in INH-induced hepatotoxicity cannot be directly established from our findings. Mitochondrial dysfunction represents another potential mechanism relevant to INH-induced hepatic injury. Mitochondria are closely involved in cellular energy metabolism and represent an important source of intracellular ROS; disruption of mitochondrial function can therefore amplify oxidative injury and hepatocellular damage (33-35). Notably, betaine has been reported to protect against acetaminophen-induced hepatotoxicity through mechanisms involving mitochondrial complex II and glutathione availability [30]. In addition, betaine has been shown to regulate mitochondrial respiration (20). These observations raise the possibility that preservation of mitochondrial function may contribute to the hepatoprotective effects observed in the present study. Nevertheless, mitochondrial bioenergetics, respiratory-chain activity, and mitochondrial membrane potential were not directly evaluated and should therefore be investigated in future studies. The present findings should also be interpreted in the context of the dose-response pattern. Betaine at 10, 50, and 100 mg/kg significantly attenuated several INH-induced biochemical and oxidative alterations. However, because the magnitude of improvement was not consistently proportional across all measured endpoints, a definitive dose-dependent effect cannot be established. Further studies incorporating formal dose-response analysis and a broader range of doses would be required to determine the optimal protective dose of betaine. Administration of betaine alone at 100 mg/kg did not significantly alter the measured serum biochemical or hepatic oxidative stress parameters compared with the control group. This finding suggests that, under the experimental conditions used, betaine did not produce detectable adverse alterations in the measured hepatic endpoints. However, this observation should not be interpreted as definitive evidence of complete safety, because a comprehensive toxicological evaluation was beyond the scope of the present study. From a translational perspective, the present findings provide experimental evidence supporting further investigation of betaine as a potential adjunctive strategy for attenuation of INH-associated hepatic injury. Previous studies have reported hepatoprotective effects of betaine in experimental models involving different hepatotoxic agents, including rifampicin, acetaminophen, and valproic acid (23, 24, 36). However, the current findings were obtained in a rat model, and the doses and experimental conditions cannot be directly extrapolated to patients receiving antitubercular therapy. Clinical studies will therefore be necessary to determine whether the hepatoprotective effects observed experimentally can be reproduced in humans. Several limitations of the present study should be acknowledged. First, the study primarily evaluated serum biochemical parameters, hepatic oxidative stress markers, and histopathological alterations. In particular, mitochondrial function, inflammatory mediators, and specific stress-responsive signaling pathways, including the Nrf2/HO-1 pathway, were not directly assessed in the present study. Therefore, although the observed changes in GSH, MDA, ROS, and FRAP support a role for altered hepatic redox homeostasis in the protective effects of betaine, the precise molecular mechanisms remain to be established. Second,

although hepatic ammonia was assessed, its mechanistic relationship with betaine-mediated hepatoprotection should not be inferred without direct assessment of urea-cycle activity or related molecular pathways. Third, the study was conducted using a single animal model and a relatively short experimental exposure, which may limit extrapolation to other species and prolonged antitubercular treatment in humans. Finally, the absence of pharmacokinetic and long-term safety assessments limits conclusions regarding the clinical applicability of betaine. Future studies incorporating mitochondrial functional assays, inflammatory mediators, antioxidant-related gene and protein expression, urea-cycle activity, specific signaling pathways, and pharmacokinetic analyses are warranted to further clarify the mechanisms and translational potential of betaine in INH-induced hepatotoxicity.

Conclusion

In conclusion, the present study demonstrates that betaine attenuated isoniazid (INH)-induced hepatic injury in rats, as evidenced by improvements in serum biochemical markers, hepatic oxidative stress parameters, and histopathological alterations. Betaine attenuated INH-induced increases in ALT, AST, ALP, LDH, total bilirubin, hepatic ROS, and MDA levels, while mitigating the reductions in serum total protein, GSH, and FRAP levels. These findings indicate that preservation of hepatic redox homeostasis may contribute to the observed hepatoprotective effect of betaine. However, the specific molecular mechanisms underlying this protection remain to be established, as mitochondrial function and related signaling pathways were not directly investigated. Overall, these findings support further preclinical investigation of betaine as a potential adjunctive strategy for limiting INH-associated hepatotoxicity, while clinical studies are required to determine its translational relevance and safety in patients receiving antitubercular therapy.

Mechanistic and Translational Relevance

The present study provides experimental evidence that betaine attenuates isoniazid (INH)-induced hepatic injury, with its protective effects accompanied by preservation of hepatic redox homeostasis and attenuation of histopathological damage. The concurrent reduction in hepatic ROS and MDA and restoration of GSH and FRAP suggest that oxidative imbalance and impaired antioxidant capacity are important components of INH-associated hepatic injury and may represent modifiable features of the hepatotoxic process. The parallel improvement in serum biochemical markers and hepatic histopathology further supports the biological relevance of this redox-associated protection. From a therapeutic perspective, these findings support further investigation of betaine as a potential adjunctive strategy for attenuating INH-associated drug-induced liver injury. However, the present findings do not establish a specific molecular target or signaling pathway responsible for betaine-mediated protection, and the observed effects cannot be directly extrapolated to clinical efficacy. Further studies integrating molecular pathway analyses, mitochondrial functional assessment, inflammatory signaling, pharmacokinetic characterization, and long-term safety evaluation are warranted to clarify the mechanisms of action and determine the translational potential of betaine in INH-associated hepatotoxicity.

Conflict of Interest

The authors declare that they have no conflict of interest.

Author Contributions

Mohsen Mohammadi and Ehsan Ahmadpouri contributed to the investigation and data curation; Amirreza Azadbakht contributed to conceptualization, methodology, and writing – original draft; Atefe Yaghoubi contributed to investigation, data curation, methodology, and histopathological analysis; and Hamidreza Mohammadi contributed to conceptualization, methodology, investigation, supervision, project administration, and writing – review & editing. All authors have read and approved the final version of the manuscript.

Ethics Statement

Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

Data Availability Statement

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Acknowledgments

The authors would like to thank the Vice Chancellor for Research and Technology of Lorestan University of Medical Sciences for supporting this research.

Funding Statement

This study was financially supported by Lorestan University of Medical Sciences, Khorramabad, Iran.

Publisher's Note

The graphical abstract was prepared by the journal's editorial team using artificial intelligence-assisted design tools and was reviewed and approved by the authors prior to publication.

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