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Harmine Attenuates Methotrexate-Induced Nephrotoxicity in Mice through Suppression of Oxidative Stress Pathways

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ABSTRACT

Although methotrexate is widely used in clinical practice, its therapeutic application is frequently restricted by adverse effects, particularly nephrotoxicity. Oxidative stress is considered a principal mechanism underlying methotrexate-induced renal injury. Harmine, a plant-derived alkaloid, exhibits notable antioxidant and anti-inflammatory properties. This study aimed to investigate the protective effects of harmine against methotrexate-induced nephrotoxicity. Mice were randomly allocated into six experimental groups: control (saline), methotrexate (20 mg/kg), harmine (20 mg/kg), and methotrexate (20 mg/kg) combined with harmine at doses of 5, 10, or 20 mg/kg. All treatments were administered intraperitoneally for 14 days. At the end of the treatment period, blood and kidney tissues were collected for biochemical, molecular, and histopathological analyses. Renal tissues were evaluated using hematoxylin–eosin (H&E) staining, quantitative real-time PCR (qRT-PCR), and oxidative stress–related biochemical assays. Methotrexate administration resulted in a significant elevation of serum creatinine and blood urea nitrogen levels, whereas treatment with harmine at doses of 10 and 20 mg/kg significantly attenuated these changes. Harmine also improved the number and diameter of glomeruli in methotrexate-treated mice. In addition, methotrexate markedly increased renal malondialdehyde and nitric oxide levels while reducing total antioxidant capacity and superoxide dismutase activity. Harmine treatment significantly reduced oxidative stress markers and restored antioxidant defense parameters. Furthermore, harmine suppressed methotrexate-induced oxidative stress by downregulating the mRNA expression of Nqo1, Ho-1, Trx1, and Nrf2. Histopathological alterations caused by methotrexate were also markedly ameliorated by harmine administration. These findings indicate that harmine exerts a protective effect against methotrexate-induced nephrotoxicity, primarily through the modulation of oxidative stress and enhancement of antioxidant defenses.

Keywords: Methotrexate, Harmine, Toxicity, Oxidative stress

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Introduction

Drug-induced nephrotoxicity represents a common adverse effect and is a major contributor to the development of acute kidney injury [1]. Methotrexate is a low–molecular weight chemotherapeutic drug that is extensively used in the treatment of various malignancies as well as inflammatory and autoimmune disorders [2–6]. However, the clinical application of methotrexate is frequently constrained by its well-documented toxicities, particularly renal injury [3, 7–9]. Because methotrexate is primarily eliminated unchanged through renal excretion [10], its administration—especially at high doses—can result in impaired renal function and, in severe cases, renal failure [2].

The nephrotoxic effects of methotrexate are thought to arise through two principal mechanisms [11]. The first involves methotrexate-induced crystalline nephropathy, in which methotrexate and its metabolites precipitate within the renal tubular lumen, leading to tubular obstruction and reduced drug clearance. This process further

compromises renal elimination, resulting in elevated systemic concentrations of methotrexate and enhanced toxicity [12]. The second mechanism is related to direct tubular injury mediated by oxidative stress, as methotrexate promotes excessive generation of reactive oxygen species (ROS) within renal tissues [13].

Cells are equipped with multiple antioxidant defense systems to maintain redox homeostasis and counteract oxidative damage [14]. Nevertheless, numerous studies have demonstrated that methotrexate disrupts these defense mechanisms by reducing antioxidant enzyme activity in the kidney. Methotrexate has been shown to increase lipid peroxidation, elevate malondialdehyde (MDA) levels, enhance myeloperoxidase (MPO) activity, and stimulate nitric oxide (NO) production in renal tissues. Concurrently, it decreases catalase activity, glutathione (GSH) content, and superoxide dismutase (SOD) activity in both blood and kidney tissues [13, 15, 16]. In addition to oxidative stress, excessive production of inflammatory mediators and infiltration of neutrophils have been implicated in methotrexate-induced renal damage [16, 17].

There is growing interest in improving the clinical utility of methotrexate through the development of adjunct therapies aimed at reducing its nephrotoxic effects. In recent years, considerable attention has been directed toward natural compounds with antioxidant and anti-inflammatory properties as potential protective agents. Several phytochemicals, including gallic acid, curcumin, caffeic acid phenethyl ester, ferulic acid, vancamine, thymoquinone, and silymarin, have demonstrated renoprotective effects against methotrexate-induced toxicity [9, 15, 17–22]. Harmine (7-methoxy-1-methyl-9H-pyrido[3,4-b]indole) is a β-carboline alkaloid found in plants such as Peganum harmala L. and is known to exhibit a range of pharmacological activities, including antimicrobial, antioxidant, and antitumor effects [23]. Previous studies have reported that harmine alleviates nicotine-induced renal damage by reducing MDA, creatinine, and NO levels [24]. Additionally, harmine has been shown to exert protective effects in lipopolysaccharide (LPS)-induced acute kidney injury by attenuating oxidative stress [25].

Based on these findings, the present study aimed to evaluate the potential protective role of harmine against methotrexate-induced nephrotoxicity and to elucidate the contribution of oxidative stress mechanisms in this drug-induced renal injury.

Materials and Methods

Ethical approval

All experimental procedures were conducted following approval from the Ethics Committee of Kermanshah University of Medical Sciences (IR.KUMS.REC.1398.1171). Animal handling and experimental protocols were performed in accordance with the Helsinki Declaration and the ethical guidelines of Kermanshah University of Medical Sciences.

Reagents and materials

Harmine (7-methoxy-1-methyl-9H-pyrido[3,4-b]indole; CAS No. 442-51-3) was purchased from Sigma. The selected dosing regimen was based on prior in vivo studies demonstrating its antioxidant and anti-inflammatory efficacy [25, 26]. Xylazine was supplied by Alfasan (Netherlands). Commercial diagnostic kits obtained from Pars Azmoon (Iran) were used for the quantification of serum creatinine and blood urea nitrogen (BUN). Total antioxidant capacity was assessed using RANDOX Total Antioxidant Status reagents (UK). Nitric oxide (NO) concentrations were determined using the Griess Reagent System (Promega). Total RNA isolation was carried out using TRIzol reagent (Invitrogen, USA), followed by cDNA synthesis using the PrimeScript First Strand cDNA Synthesis Kit (TaKaRa Bio, Japan). Quantitative gene expression analysis was performed with SYBR Green Master Mix (TaKaRa Bio Inc., Japan).

Animals and treatment design

A total of 42 male BALB/c mice (27–30 g) were housed under standard laboratory conditions, including a controlled temperature of 23 ± 2 °C and a 12-h light/dark cycle, with unrestricted access to food and water. Animals were randomly allocated into six experimental groups (n = 7 per group): saline-treated control; methotrexate-treated (20 mg/kg); harmine-treated (20 mg/kg); and methotrexate (20 mg/kg) combined with harmine at doses of 5, 10, or 20 mg/kg.

All treatments were administered intraperitoneally once daily over a 14-day period. Normal saline served as the vehicle, and all solutions were freshly prepared before injection. On the fifteenth day, 24 h after the final administration, animals were anesthetized with ketamine (70 mg/kg) and xylazine (10 mg/kg). Blood samples

were collected via the abdominal aorta and centrifuged at 3,000 rpm for 15 min at 4 °C to obtain serum. Kidneys were rapidly excised; one portion was processed for histopathological evaluation, while the remaining tissue was snap-frozen in liquid nitrogen and stored at −80 °C for molecular analysis.

Histological examination

Renal tissues were immersed in 10% buffered formalin for fixation over 24 h. Each kidney was longitudinally sectioned through the center prior to paraffin embedding. Serial tissue sections (5–7 μm thick) were prepared and stained using hematoxylin and eosin (H&E). Microscopic evaluation was performed using a light microscope (Olympus CH3, Japan). Quantitative morphometric analysis included measurement of glomerular diameter in 100 randomly selected glomeruli using DP2-BSW software. In addition, glomerular density was determined by counting glomeruli in randomly chosen microscopic fields and calculating the average.

Assessment of renal function

Serum creatinine and blood urea nitrogen levels were measured using standardized commercial assay kits in accordance with the manufacturers' protocols.

Evaluation of oxidative stress and antioxidant capacity

Superoxide dismutase (SOD) activity and malondialdehyde (MDA) concentrations were determined according to the methods described by Nishikimi *et al*. and Ohkawa *et al*., respectively [27, 28]. Total antioxidant capacity was measured using a Total Antioxidant Status assay. Renal nitric oxide levels were quantified using the Griess reaction based on the protocol described by Giustarini *et al*. [29].

Quantitative real-time PCR analysis

Total RNA was extracted from frozen kidney tissues using TRIzol reagent. Complementary DNA was synthesized from isolated RNA using the PrimeScript First Strand cDNA Synthesis Kit. Quantitative real-time PCR was conducted using SYBR Green chemistry on a Corbett Rotor-Gene 6000 system. Relative gene expression levels were calculated using the comparative Ct method following normalization to β-actin and were expressed as fold changes relative to the control group. Primer sequences employed in the analysis are presented in **Table 1.**

Table 1. Primer sequences used for quantitative real-time PCR.

Primer	Sequence
Nrf2	Backward: 5′-TGAGACACTGGTCACACT-3′ Forward: 5′-CAGCATGATGGACTTGGA-3′
Ho-1	Backward: 5′-GCAGCTCCTCAAACAGCTCAA-3′ Forward: 5′-CCTTCCCGAACATCGACAGCC-3′
Trx1	Forward: 5′-CCCTTCTTCCATTCCCTCT-3′Backward: 5′-TCCACATCCACTTCAAGGAAC-3′
Nqo1	Forward: 5′-AAGGATGGAAGAAACGCCTGGAGA-3′Backward: 5′-GGCCCACAGAAAGGCCAAATTTCT-3′

Statistical analysis

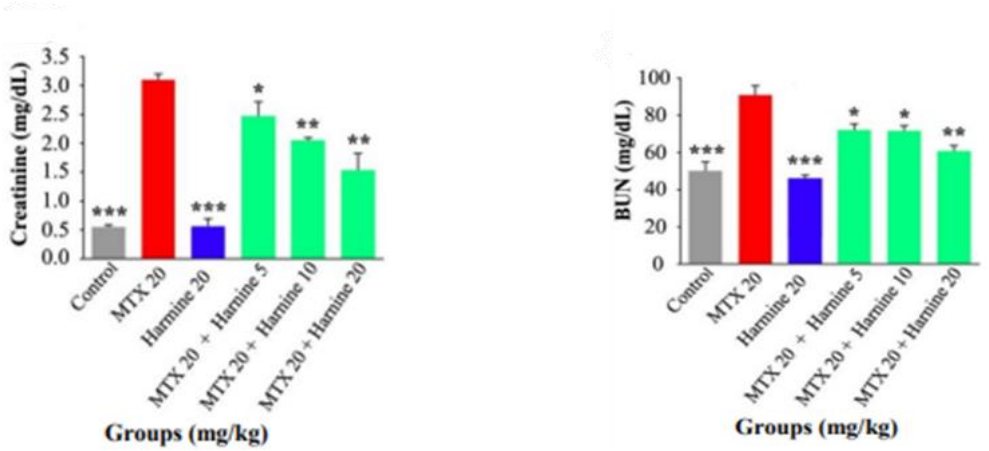
Data analysis was carried out using SPSS software version 19.0 (SPSS Inc., Chicago, IL, USA). Group comparisons were conducted using one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test. All data are presented as mean ± standard error of the mean (SEM). A probability value of less than 0.05 was considered indicative of statistical significance.

Results and Discussion

The findings of the present study demonstrate that harmine administration effectively attenuated methotrexate-induced renal injury in mice, primarily through enhancement of antioxidant defense mechanisms and modulation of genes associated with oxidative stress responses. Methotrexate, a widely used chemotherapeutic agent, is known to induce renal toxicity, which significantly limits its therapeutic applicability [11]. Previous investigations have highlighted the beneficial effects of various phytochemicals possessing intrinsic antioxidant properties in reducing methotrexate-induced nephrotoxicity by reinforcing endogenous antioxidant systems [9, 17, 19, 20, 22]. Harmine, the bioactive compound examined in this study, is a naturally occurring antioxidant with diverse pharmacological activities, including renoprotective effects [25, 26]. Earlier studies have reported that harmine alleviates dioxin-induced liver injury [30] and confers protection against lipopolysaccharide (LPS)-induced acute

kidney damage [25]. However, its potential protective or therapeutic role in methotrexate-associated renal toxicity has not previously been explored. In this study, we therefore evaluated the capacity of harmine to mitigate methotrexate-induced kidney injury in a murine model.

Existing evidence indicates that methotrexate-induced nephrotoxicity is characterized by elevated serum creatinine and blood urea nitrogen levels, along with manifestations such as uremia and hematuria [9, 31].



a) b)

Figure 1. Changes in renal function markers following administration of methotrexate (20 mg/kg) and harmine (5, 10, and 20 mg/kg). Results are shown as mean ± SEM (n = 7). Statistical significance relative to the methotrexate group is indicated by *p < 0.05, **p < 0.01, and ***p < 0.001. Abbreviations: BUN, blood urea nitrogen; MTX 20, methotrexate (20 mg/kg); harmine 5, 10, and 20, harmine at corresponding doses.

Exposure to methotrexate resulted in a pronounced deterioration of renal function, as evidenced by a significant elevation in serum creatinine and BUN concentrations when compared with untreated controls ($p < 0.001$); **(Figures 1a and 1b).** In contrast, co-administration of harmine at doses of 10 and 20 mg/kg markedly attenuated these increases, demonstrating a dose-dependent improvement in kidney function ($p < 0.05$ and $p < 0.01$, respectively).

Histopathological examination corroborated the biochemical findings. Renal sections obtained from control animals exhibited preserved glomerular morphology and intact tubular organization **(Figure 2a).** In sharp contrast, kidneys from methotrexate-treated mice displayed extensive structural disruption, including epithelial desquamation, tubular lumen expansion, vascular congestion, fibrotic alterations within glomeruli, and cytoplasmic vacuole formation **(Figure 2b).** Treatment with harmine alone (20 mg/kg) did not induce detectable histological abnormalities and closely resembled the control morphology **(Figure 2c).** Importantly, concurrent administration of harmine (20 mg/kg) with methotrexate substantially mitigated these pathological changes, indicating significant preservation of renal tissue integrity **(Figure 2d).** Quantitative histopathological findings are summarized in **Table 2.**

Morphometric assessment further revealed that methotrexate exposure significantly reduced both glomerular density and glomerular diameter ($p < 0.001$ and $p < 0.01$, respectively). These structural impairments were significantly reversed in mice receiving harmine alongside methotrexate, as demonstrated by restored glomerular number and size **(Figures 2e and 2f).**

Accumulating evidence identifies oxidative stress, together with inflammatory signaling, as a central contributor to methotrexate-induced renal injury [8, 9, 13, 22]. Excessive generation of reactive oxygen species (ROS), depletion of endogenous antioxidant systems, and enhanced lipid peroxidation are consistently reported features of methotrexate toxicity in multiple organs, including the kidney [8, 9]. The elevated ROS burden associated with methotrexate has been attributed to mechanisms such as neutrophil recruitment and activation [32], impairment of mitochondrial function [33], and increased NADPH oxidase activity [34]. Moreover, ROS overproduction activates downstream inflammatory cascades involving nuclear factor-κB (NF-κB), tumor necrosis factor-α (TNFα), and several interleukins, including IL-1β and IL-10 [21, 35], all of which play critical roles in renal damage progression [21].

Given that suppression of oxidative stress and reinforcement of antioxidant defenses can significantly alleviate methotrexate-induced nephrotoxicity [19, 21], harmine was evaluated for its antioxidative efficacy. To this end, total antioxidant capacity (TAC), malondialdehyde (MDA), and superoxide dismutase (SOD) were measured as indicators of redox status **(Figure 3).** Methotrexate administration led to a marked reduction in TAC levels relative to controls ($p < 0.001$). Notably, treatment with harmine at all tested doses significantly restored antioxidant capacity **(Figure 3a).**

Consistent with enhanced oxidative damage, methotrexate markedly increased renal MDA levels compared with control animals ($p < 0.001$). However, harmine administration at doses of 10 and 20 mg/kg significantly suppressed lipid peroxidation, as reflected by reduced MDA concentrations ($p < 0.05$); **(Figure 3b).** These observations align with previous findings by Salahshoor *et al*., who reported normalization of MDA, BUN, creatinine, and NO following harmine treatment in a nicotine-induced renal injury model [24].

In parallel, methotrexate exposure significantly diminished SOD activity after 14 days of treatment ($p < 0.001$). Co-treatment with harmine at 20 mg/kg effectively restored SOD activity toward control levels ($p < 0.01$); **(Figure 3c).** Similar oxidative alterations following methotrexate exposure have been reported by Hassanein *et al*. in a lung injury model [22]. Additionally, Niu *et al*. demonstrated that methotrexate reduced glutathione (GSH) content and SOD activity across hepatic, renal, and cardiac tissues. Notably, harmine pretreatment (25 or 50 mg/kg) significantly alleviated LPS-induced acute kidney injury by decreasing MDA and MPO levels while enhancing SOD and GSH activity [25].

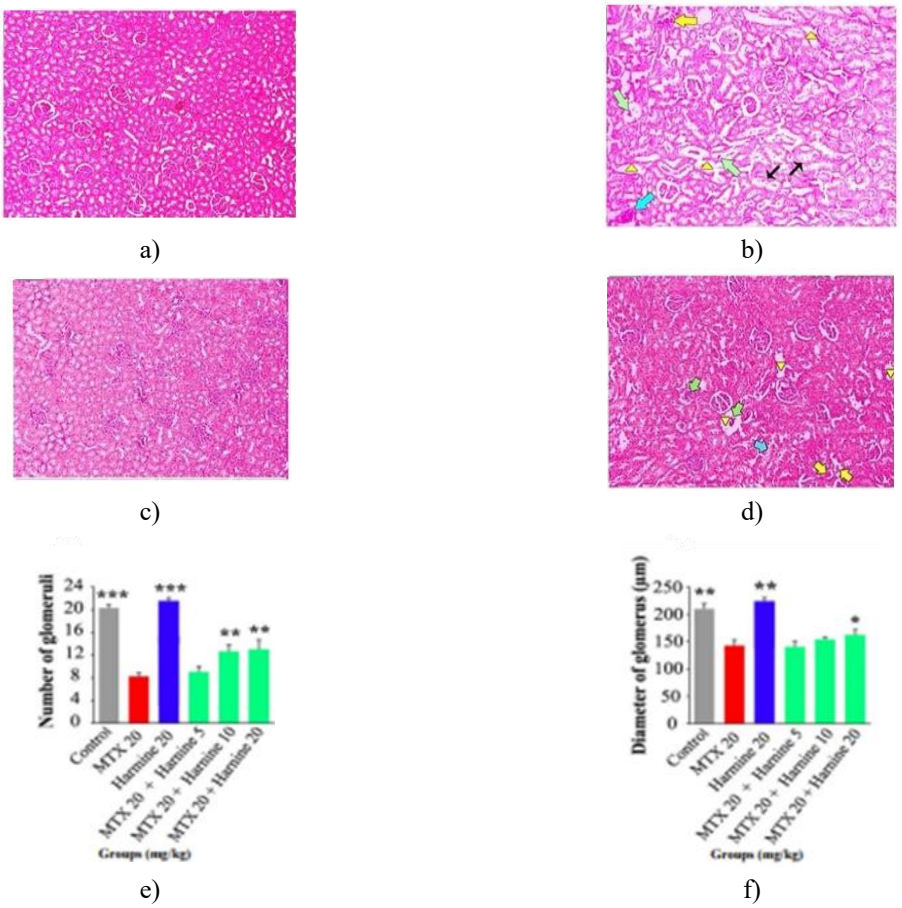


Figure 2. Representative renal histological findings following treatment with methotrexate (20 mg/kg) and harmine (5, 10, and 20 mg/kg). Kidney sections were stained with hematoxylin and eosin (H&E). (a) Control group showing normal renal architecture. (b) Methotrexate-treated mice exhibiting proteinaceous casts, detachment of tubular epithelial cells, intracellular vacuole formation, glomerular fibrosis, and dilation of renal tubules (indicated by arrows and triangles). (c) Harmine-treated group (20 mg/kg) displaying preserved renal morphology. (d) Combined treatment with methotrexate (20 mg/kg) and harmine (20 mg/kg) demonstrating marked attenuation of histopathological abnormalities. (e) Quantitative analysis of glomerular number. (f) Measurement of glomerular diameter (µm). Data are presented as mean ± SEM; *$p < 0.05$, **$p <$

0.01, ***p < 0.001 versus methotrexate-treated group. MTX 20: methotrexate (20 mg/kg); harmine 5, 10, and 20: harmine administered at doses of 5, 10, and 20 mg/kg, respectively.

Methotrexate administration led to a pronounced elevation in renal nitric oxide (NO) levels compared with the control group ($p < 0.001$). Although harmine treatment reduced NO concentrations across all doses, a statistically significant reduction was observed only at the highest dose (20 mg/kg; $p < 0.001$); **(Figure 3d).** Nitric oxide plays a critical role in the development of acute renal injury, as its free radical nature contributes to tubular epithelial damage [34]. Furthermore, NO reacts with superoxide anions to form peroxynitrite, a highly reactive and cytotoxic species that exacerbates tubular injury and promotes renal dysfunction [35]. The observed decrease in NO following harmine treatment may be attributed to suppression of inducible nitric oxide synthase (iNOS) expression [36]. Activation of nuclear factor-kappaB (NF-κB) by reactive oxygen species (ROS), followed by upregulation of iNOS, is considered a principal mechanism underlying excessive NO production during methotrexate-induced toxicity [8].

Consistent with this mechanism, co-treatment with harmine significantly lowered NO levels compared with mice receiving methotrexate alone. Similar antioxidant-mediated effects have been reported by Morsy *et al*., who demonstrated that curcumin markedly reduced lipid peroxidation and NO elevation in methotrexate-intoxicated animals. In addition, curcumin significantly enhanced renal glutathione peroxidase and superoxide dismutase (SOD) activities relative to the methotrexate-treated group [18].

To further elucidate the molecular mechanisms responsible for the protective effects of harmine, renal mRNA expression levels of heme oxygenase-1 (Ho-1), nuclear factor erythroid 2–related factor 2 (Nrf2), NAD(P)H:quinone oxidoreductase 1 (Nqo1), and thioredoxin 1 (Trx1) were quantified using qRT-PCR. As illustrated in **Figure 4**, methotrexate administration (20 mg/kg) caused a significant suppression of Ho-1, Nrf2, Nqo1, and Trx1 gene expression in kidney tissues ($p < 0.001$). The immune and cellular defense systems can modulate the expression of cytoprotective enzymes in response to harmful stimuli. Among these regulatory mechanisms, Nrf2 serves as a master transcription factor governing the expression of genes involved in cellular defense against oxidative and electrophilic stress [37].

Previous studies have highlighted the essential role of Nrf2 in maintaining renal homeostasis and protecting kidney tissue from various pathological insults [28, 38]. Activation of the Nrf2 pathway promotes detoxification and elimination of reactive oxygen species, processes that are critically important in mitigating renal toxicity [28]. Consequently, pharmacological activation of Nrf2 has been proposed as a promising therapeutic strategy for renal disorders. Supporting this concept, a large multicenter clinical trial demonstrated improved renal outcomes in patients with type II diabetes and chronic kidney disease following treatment with bardoxolone methyl, a potent Nrf2 activator [39].

In the present study, methotrexate exposure resulted in a marked reduction of renal Nrf2 expression in intoxicated mice ($p < 0.001$). In contrast, administration of harmine at a dose of 20 mg/kg significantly restored Nrf2 expression levels ($p < 0.001$). It is well established that genes encoding thioredoxin reductase 1 (TXNRD1), NAD(P)H:quinone oxidoreductase 1 (Nqo1), and heme oxygenase-1 (Ho-1) are transcriptionally regulated through Nrf2 binding. Activation of this signaling cascade enhances cellular resistance to oxidative stress and confers robust cytoprotection [40].

Consistent with these findings, Mahmoud *et al*. reported that methotrexate-induced oxidative stress suppressed Nrf2 expression and nuclear translocation, leading to downregulation of downstream antioxidant genes such as Ho-1 and Nqo1. Their study further demonstrated that treatment with the antioxidant ferulic acid activated the renal Nrf2/HO-1 signaling pathway, which was associated with improved antioxidant capacity and reduced renal injury [9].

Table 2. Histopathological grading of renal tissue alterations following methotrexate and harmine administration in mice.

Experimental group	Cytoplasmic vacuole formation	Tubular lumen enlargement	Detachment of tubular epithelium	Renal vascular congestion	Fibrotic changes in glomeruli
Methotrexate (20 mg/kg)	3	3	2	4	3
Untreated control	1	1	1	1	0
Methotrexate (20 mg/kg) + harmine (5 mg/kg)	2	3	2	4	2

Harmine alone (20 mg/kg)	1	1	1	1	0
Methotrexate (20 mg/kg) + harmine (20 mg/kg)	1	1	1	2	1
Methotrexate (20 mg/kg) + harmine (10 mg/kg)	1	2	1	2	1

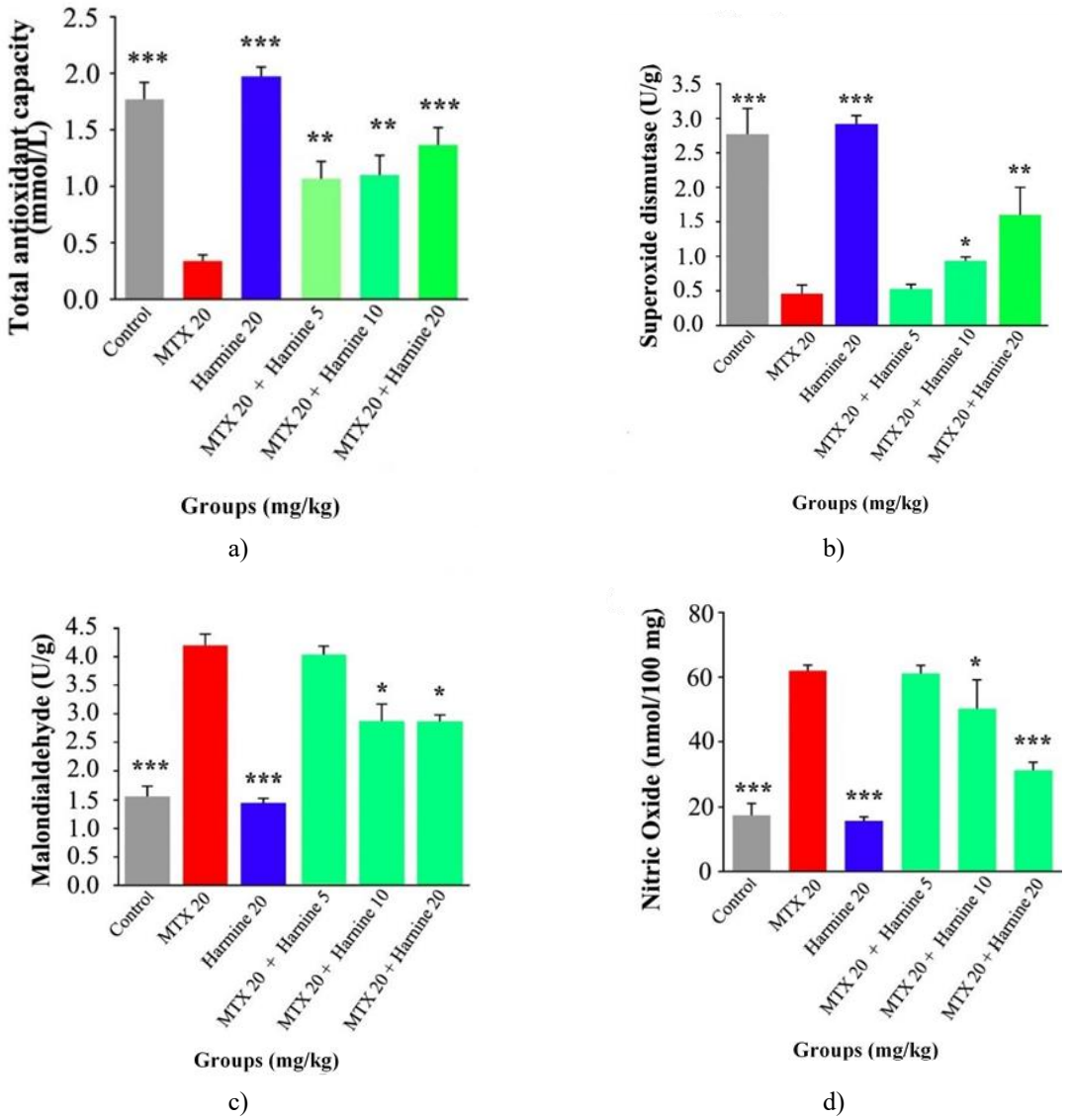


Figure 3. Influence of harmine (5, 10, and 20 mg/kg) and methotrexate (20 mg/kg) on renal oxidative stress indicators in mice. Values are presented as mean ± SEM (n = 7). Statistical significance: *p<0.05; **p<0.01; ***p<0.001 versus the methotrexate-treated group. MDA, malondialdehyde; TAC, total antioxidant capacity; MTX 20, methotrexate (20 mg/kg); SOD, superoxide dismutase; harmine 5, 10, 20 correspond to harmine doses of 5, 10, and 20 mg/kg, respectively.

Heme oxygenase-1 (HO-1) is upregulated in multiple experimental models of renal disorders and serves as a key cytoprotective enzyme against oxidative damage [40, 41]. In the present study, methotrexate administration resulted in a marked reduction in renal Ho-1 mRNA levels. In contrast, treatment with harmine at 20 mg/kg significantly enhanced Ho-1 gene expression ($p<0.001$). Moreover, combined administration of harmine and methotrexate significantly restored Ho-1 expression relative to methotrexate alone ($p<0.01$), indicating a protective regulatory effect.

NAD (P) H quinone dehydrogenase 1 (Nqo1) is rapidly induced in response to diverse cellular stressors, particularly oxidative challenges. In both humans and mice, Nqo1 transcription is primarily governed by

antioxidant response elements (AREs) located within its promoter region, playing a pivotal role in maintaining redox balance and cellular adaptation to oxidative stress [42]. Activation of Nrf2 is known to strongly stimulate Nqo1 expression [43]. Our findings demonstrated a significant elevation of Nqo1 transcript levels in harmine-treated mice ($p<0.001$), while the suppressive effect of methotrexate on Nqo1 expression was significantly attenuated following harmine co-treatment ($p<0.05$).

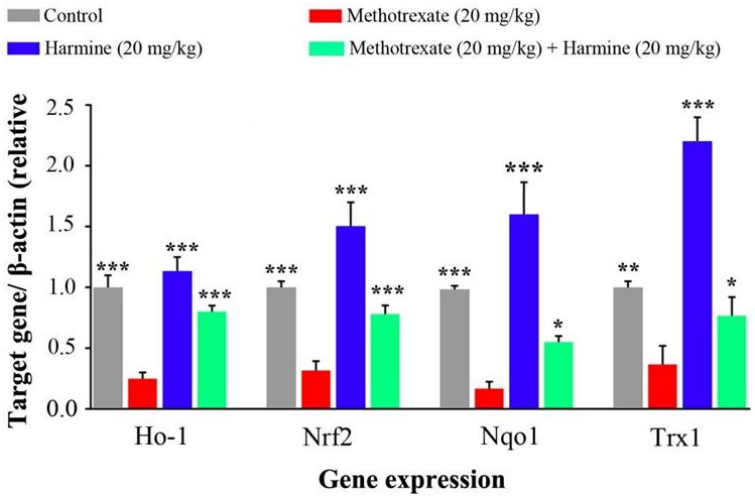


Figure 4. Effects of harmine (5, 10, and 20 mg/kg) and methotrexate (20 mg/kg) on renal expression of oxidative stress–related genes in mice. Data are expressed as mean ± SEM (n = 7). *p<0.05; **p<0.01; ***p<0.001 compared with the methotrexate group. NRF2, nuclear factor erythroid 2–related factor 2; Ho-1, heme oxygenase-1; Nqo1, NAD (P) H quinone dehydrogenase 1; TRX1, thioredoxin 1.

Thioredoxin 1 (Trx1) is a ubiquitously expressed antioxidant protein that plays a crucial role in intracellular redox regulation and neutralization of reactive oxygen species (ROS) [44]. Previous investigations have shown that oxidative stress, such as that induced by ischemic injury, promotes Trx secretion from renal tubular epithelial cells into the urine [45]. The interaction between Nrf2 signaling and the thioredoxin system suggests that Nrf2-driven antioxidant responses reduce ROS accumulation and preserve the balance between thioredoxin and thioredoxin-interacting protein (TXNIP), ultimately suppressing ROS-dependent activation of NF-κB signaling pathways [46]. In this study, co-treatment with harmine and methotrexate resulted in a significant elevation of renal Trx1 expression, consistent with reduced oxidative burden mediated by harmine. Methotrexate alone markedly downregulated Trx1 expression in kidney tissue ($p<0.001$), whereas harmine administration significantly counteracted this reduction in the co-treatment group ($p<0.05$). Additionally, mice receiving harmine alone exhibited a pronounced increase in Trx1 mRNA expression compared to controls ($p<0.001$).

The protective effects of natural and synthetic compounds in experimental tissue injury models have largely been attributed to their antioxidant properties [47–51]. Another potential mechanism underlying harmine's protective action may involve modulation of systemic blood pressure [52]. However, in the current study, no direct evidence was obtained to support a role for blood pressure regulation in harmine-mediated renal protection. Consequently, future investigations should include blood pressure assessment to clarify its contribution in kidney injury models.

Conclusion

Methotrexate administration promoted pronounced oxidative damage in the renal tissue of mice, whereas harmine, a naturally occurring antioxidant compound, effectively counteracted methotrexate-induced excessive generation of reactive oxygen species and strengthened endogenous antioxidant defense mechanisms. These findings suggest that harmine has considerable potential as a protective therapeutic agent for reducing methotrexate-associated kidney toxicity.

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Conflict of Interest: None

Financial Support: None

Ethics Statement: None

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