RESEARCH ARTICLE Indian Journal of Animal Research, Volume 59 Issue 10: 1681-1689 (October 2025)

Evaluation of the Hepatoprotective Role of Diosmin in a Rat Model of Thioacetamide-induced Liver Fibrosis

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10.18805/IJAR.BF-2010

ABSTRACT

Background: Liver fibrosis is a progressive and potentially life-threatening condition characterized by excessive extracellular matrix accumulation that impairs hepatic function. Diosmin, a citrus-derived flavonoid, has shown promising antioxidant and anti-inflammatory properties in various models. This study aimed to evaluate the protective effects of diosmin against thioacetamide (TAA)-induced liver fibrosis in rats.

Methods: Forty male Wistar rats were randomly assigned to four groups (n=10 each): control, TAA (200 mg/kg, intraperitoneally, three times per week), diosmin (50 mg/kg/day, oral) and TAA + diosmin. Treatments lasted for 6 weeks. Liver function, oxidative stress, inflammation and apoptosis were assessed using standard biochemical assays, ELISA and histological evaluation with hematoxylin-eosin staining and Masson's trichrome.

Result: TAA significantly impaired liver function, indicated by increased AST, ALT and ALP levels and decreased albumin. It also induced oxidative stress, reflected by increased NO and MDA levels and decreased levels of GSH, GPx, GR, SOD and CAT. Inflammatory markers including IL-1β, TNF-α and NF-κB were elevated, while apoptotic indicators showed an increased Bax/Bcl-2 ratio and enhanced caspase-3 activity. Diosmin co-treatment improved liver function, restored antioxidant status, reduced inflammatory mediators and regulated apoptotic markers. Histological findings supported these results by showing reduced fibrosis and preserved liver architecture. These findings suggest that diosmin effectively protects against thioacetamide-induced liver fibrosis through its antioxidant, anti-inflammatory and anti-apoptotic properties, warranting further investigation as a potential therapeutic agent for liver fibrosis.

Key words: Antioxidants, Diosmin, Fibrosis, Inflammation, Oxidative stress, Thioacetamide.

INTRODUCTION

Thioacetamide is metabolized in hepatic tissue by cytochrome P450 enzymes to its active metabolite thioacetamide-S-oxide, which interacts with cellular macromolecules leading to hepatic injury (Chen *et al.,* 2019). The hepatotoxic effects of thioacetamide are primarily mediated through the generation of reactive oxygen species (ROS) and induction of oxidative stress in hepatic tissue (Staňková *et al.,* 2010).

Thioacetamide induces liver fibrosis in experimental models by triggering oxidative damage and inflammatory responses, leading to activation of Kupffer cells and release of pro-inflammatory cytokines including IL-1β and TNF-α (Mi *et al.,* 2019). Subsequently, hepatic stellate cells (HSCs) are activated through increased oxidative stress and inflammatory mediators, causing their transformation into myofibroblasts that produce excessive collagen, resulting in liver fibrosis (Zhang *et al.,* 2016). If left untreated, liver fibrosis can progress to cirrhosis with severe complications.

Oxidative stress and inflammation promote hepatocyte apoptosis *via* mitochondrial pathways (Wang, 2014). Elevated ROS disrupt mitochondrial function and shift the Bax/Bcl-2 balance, increasing Bax and reducing Bcl-2, which facilitates cytochrome c release and caspase-3 activation. Thus, oxidative stress acts as a key trigger for liver injury, enhancing inflammation and fibrosis progression (Khajuria *et al.,* 2018).

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How to cite this article: Albrakati, A. (2025). Evaluation of the Hepatoprotective Role of Diosmin in a Rat Model of Thioacetamide-induced Liver Fibrosis. *Indian Journal of Animal Research*. **59(10):** 1681-1689. doi: 10.18805/IJAR.BF-2010.

Submitted: 14-04-2025 **Accepted:** 21-06-2025 **Online:** 10-07-2025

Recent studies have shown that enhanced hepatocyte apoptosis contributes significantly to the progression of hepatic fibrosis through multiple mechanisms (Cheng *et al.,* 2024). Apoptotic hepatocytes release damage-associated molecular patterns that activate Kupffer cells and promote inflammation (Ju and Tacke, 2016). Additionally, the engulfment of apoptotic bodies by hepatic stellate cells triggers their activation and transformation into myofibroblasts, leading to increased collagen deposition and fibrosis progression. This creates a self-perpetuating cycle where inflammation and oxidative stress promote apoptosis, which in turn enhances fibrogenic responses (Antar *et al.,* 2023).

Nrf2 is a key transcription factor in cellular antioxidant defense. Under normal conditions, it remains bound to Keap1 in the cytoplasm (He *et al.,* 2020). Oxidative stress

triggers modifications in Keap1, leading to the release and nuclear translocation of Nrf2. In the nucleus, Nrf2 binds to antioxidant response elements (AREs), activating genes encoding antioxidant enzymes such as GPx, SOD and CAT (Yu and Xiao, 2021). These enzymes work synergistically to neutralize reactive oxygen species (ROS), forming a vital defense mechanism against oxidative damage and fibrosis progression (Jomova *et al.,* 2024).

Primarily from citrus fruits, diosmin is a naturally occurring flavonoid glycoside with a flavone nucleus linked to a disaccharide (Naso *et al.,* 2016). It has demonstrated antioxidant, anti-inflammatory and vasoprotective properties in numerous pathological conditions (Hassanein *et al.,* 2024). Mechanistically, diosmin scavenges reactive oxygen species (AlAsmari *et al.,* 2024), inhibits lipid peroxidation and enhances antioxidant enzymes including glutathione-related systems (Wójciak *et al.,* 2022). Its anti-inflammatory effects involve blocking NF-κB signalling and reducing pro-inflammatory cytokines (Shaaban *et al.,* 2022). Therapeutic applications include vascular diseases (Feldo *et al.,* 2019), inflammatory conditions (Sukalingam *et al.,* 2018), oxidative stress-induced organ damage (Taylor *et al.,* 2022) and diabetic neuropathy (Jain *et al.,* 2014). Thus, by looking at several biochemical, molecular and histological factors, this study aimed to explore the possible protective effects of diosmin against thioacetamide-induced liver fibrosis in rats.

MATERIALS AND METHODS

Chemicals and reagents

All chemicals and reagents used in this study were of analytical grade. Thioacetamide (Cat 163678) and diosmin 3′,5,7-Trihydroxy-4′-methoxyflavone 7-rutinoside (CAS Number: 520-27-4) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Other chemicals were also obtained from Sigma-Aldrich. The chemical structure of diosmin is shown in Fig 1.

Animals and experimental design

Male wistar rats (180-200 g, 8-10 weeks old) were housed under standard conditions (22±2°C, 55±5% humidity, 12-h light/dark cycle). Animals received ad libitum Envigo Rodent Diet 2018SX (18% protein, 5% fat, 4% fiber, 8% ash, 10% moisture, 55% NFE) and purified tap water (pH 7.2).

Following one week of acclimatization, rats were randomly divided into four groups (n=10 per group): Control group received 0.9% saline only; TAA group received thioacetamide (200 mg/kg, intraperitoneally, three times per week for six weeks) (Al-Malki, 2019); Diosmin group received diosmin (50 mg/kg/day, oral gavage for six weeks) (Wang *et al.,* 2024) and TAA+Diosmin group received both TAA and diosmin. The years during which the experiments were performed (2019-2020).

Biochemical analyses

Liver function tests

Serum alanine transaminase, alkaline phosphatase and aspartate transaminase activities Liver function enzymes (ALT, AST and ALP) were assessed according to Reitman and Frankel (1957). Serum albumin levels were measured using the bromocresol green method (Doumas *et al.,* 1971).

Oxidative stress markers

Hepatic nitric oxide (NO) levels were determined using the Griess reaction method (Robbins *et al.,* 1996). (MDA) levels were determined the procedure established by Ohkawa *et al.* (1979). Reduced glutathione (GSH) content was determined using the method outlined by Ellman (1959).

Antioxidant enzyme activities

The activity of hepatic superoxide dismutase (SOD) was assessed using the technique described by Nishikimi *et al.* (1972). The level of hepatic glutathione reductase (GR,) was assessed using the technique described by Moron *et al.* (1979). Catalase activity was determined using the technique described by Aebi (1984). Glutathione peroxidase (GPx) activity was assessed using the technique described by Paglia and Valentine (1967). The protein content of the liver was assessed using the methodology established by Lowry *et al.* (1951). Nuclear factor erythroid 2-related factor 2 (Nrf2) levels were quantified according to the protocols documented by Bradford (1976).

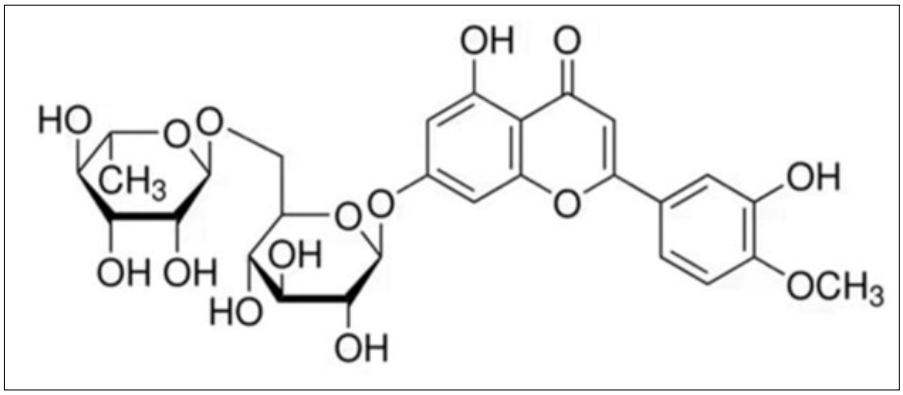


Fig 1: Chemical structure of diosmin (Sigma-Aldrich).

Inflammatory markers

Liver tissue levels of inflammatory mediators including TNF-α, IL-1β and NF-κB were measured using specific ELISA kits according to manufacturer's protocols.

Apoptotic markers

Liver tissue levels of Bcl-2 and Bax were measured using ELISA kits. Caspase-3 activity was assessed using a colorimetric kit. All assays were conducted according to manufacturer's instructions.

Histopathological examination

Liver tissues fixed in 10% neutral buffered formalin were processed through ascending grades of alcohol (70-100%, 2 h each), cleared in xylene (2 changes, 1 hour each) and embedded in paraffin wax. Sections of 5 μm thickness were cut using a rotary microtome (Leica RM2255, Germany) and stained with hematoxylin and eosin (H andE) for general histological examination. Additional sections were stained with Masson's trichrome for visualization of collagen fibers. All stained sections were examined qualitatively by a single blinded pathologist under a light microscope (Olympus BX53) (Cardiff *et al.,* 2014).

Ethical approval

This study, along with all experimental procedures, was conducted according to the principles outlined by the ethics committee of the University of Taif (Approval No. HAPO-O2-T-105).

Statistical analysis

All data are expressed as mean±SEM. Statistical analysis was conducted using GraphPad Prism (version-9.0). One-way ANOVA followed by Tukey's post-hoc test was used for group comparisons. Pearson's correlation was applied and $p<0.05$ was considered statistically significant. All assays were performed in triplicate.

RESULTS AND DISCUSSION

Evaluation of hepatic enzymatic functions

Liver markers were significantly altered ($p<0.05$). Albumin was significantly lower in the TAA group (1.7±0.1 mg/dl), smaller than control (3.0/ ±0.2) and improved with diosmin (2.8±0.1) ($p<0.05$). AST, ALT and ALP were significantly higher in TAA (74±6, 160±18, 275±8), greater than control (42±2, 108±10, 125±5) and were reduced with diosmin (49±1, 122±5, 165±10) ($p<0.05$), indicating hepatoprotective effects (Fig 2).

Oxidative stress parameters analysis

Oxidative stress markers were significantly altered among the study groups. No levels were significantly elevated ($p<0.05$) in the TAA group (~160 pmol/g) compared to the control (~110), but were reduced ($p<0.05$) following diosmin co-treatment (~120). Similarly, MDA levels increased markedly ($p<0.05$) in the TAA group (~220 nmol/g) versus the control (~140) and decreased to (~150) with diosmin. In contrast, GSH levels were significantly depleted ($p<0.05$) in the TAA group (~0.6 mg/dl) compared to control (~1.7), while diosmin supplementation restored levels (~1.5 mg/dl), suggesting a reduction in oxidative damage (Fig 3).

Antioxidant enzyme activities

Significant differences in antioxidant enzyme activities were observed across treatment groups. The control group showed normal physiological levels: CAT (1.75±0.2 U/mg), GPx (1.25±0.12 U/mg), GR (0.95±0.08 μmol/mg) and SOD

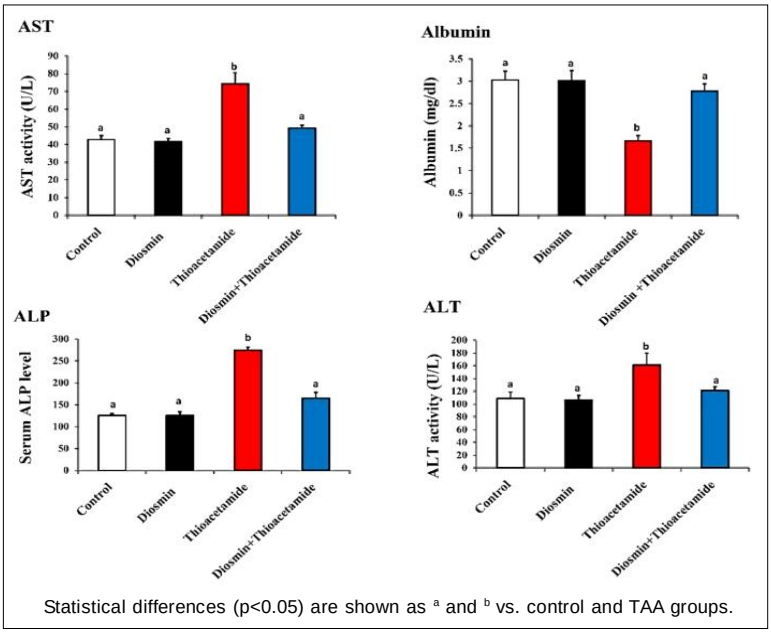


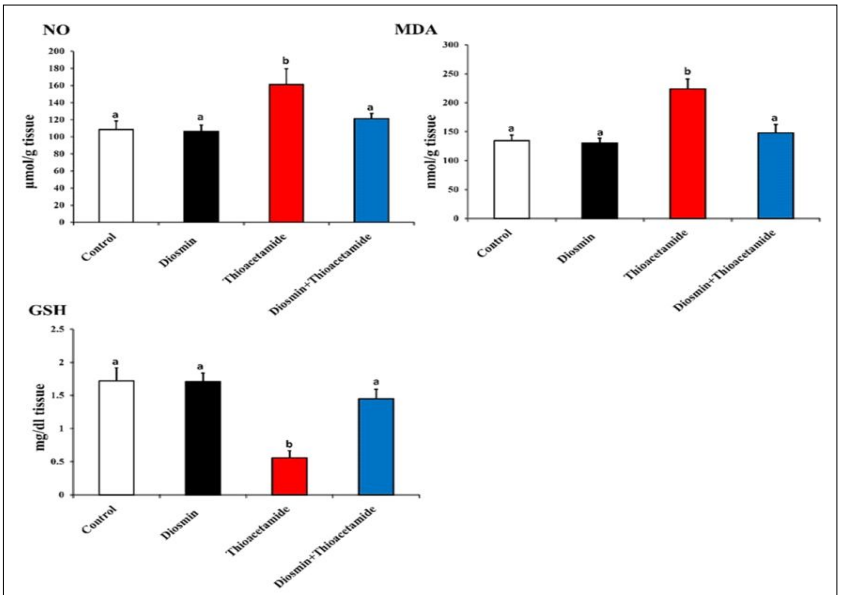
Fig 2: Serum levels of AST, ALT, ALP and albumin.

(5.8±0.8 U/mg). These values remained unchanged in the diosmin-only group, indicating no adverse effect. Thioacetamide significantly impaired antioxidant defenses ($p<0.05$), reducing CAT to 0.7±0.2, GPx to 0.54±0.08, GR to 0.45±0.12 and SOD to 3.1±0.5, indicating marked oxidative damage.

Co-treatment with diosmin significantly restored enzyme activities: CAT (1.55±0.1), GPx (1.15±0.17), GR (0.85±0.10) and SOD (5.5±0.7), approaching control levels. Additionally, Nrf-2 expression, which dropped to 0.4±0.05 pg/g in the thioacetamide group, was elevated to 0.83±0.12 pg/g following diosmin treatment, reflecting reactivation of antioxidant signaling. These findings confirm diosmin's ability to restore enzymatic antioxidant defenses and enhance Nrf-2-mediated protection (Fig 4).

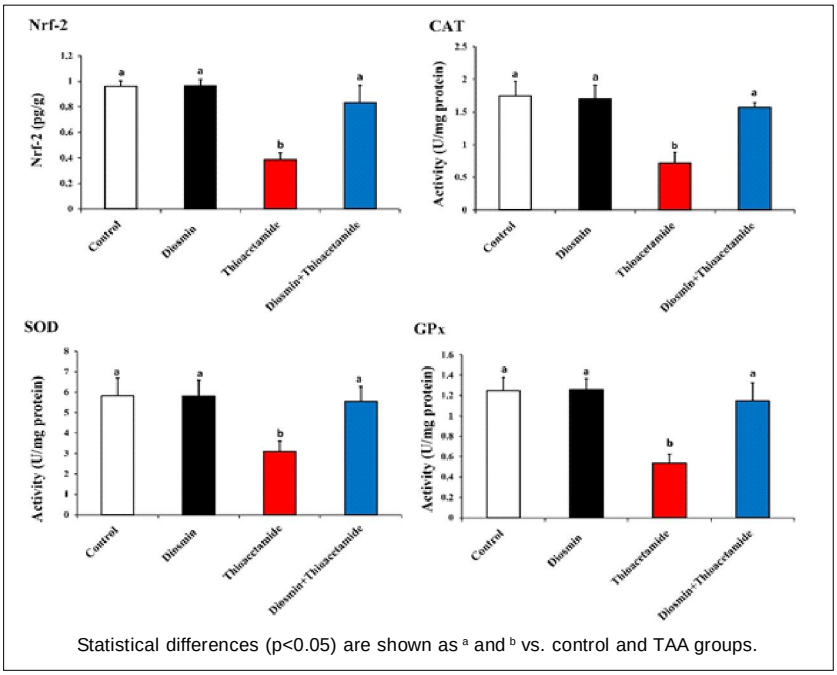
Anti-inflammatory markers

Analysis of inflammatory mediators revealed significant elevations in the thioacetamide group ($p<0.05$), indicating



Statistical differences ($p<0.05$) are shown as [a] and [b] vs. control and TAA groups.

Fig 3: Hepatic levels of oxidative stress parameters analysis.



Statistical differences ($p<0.05$) are shown as [a] and [b] vs. control and TAA groups.

Fig 4: Hepatic levels of antioxidant enzyme activities.

strong inflammatory activation. IL-1β levels increased to 2.2±0.3 pg/mg protein ($p<0.05$)-greater than control (1.1±0.1). Similarly, NF-κB and TNF-α levels rose to 6.3±0.4 and 3.7±0.3 pg/mg ($p<0.05$), respectively-greater than control values (2.3±0.2 and 2.1±0.1). In contrast, the diosmin-only group maintained values comparable to control: IL-1β (1.1±0.2), NF-κB (2.2±0.1) and TNF-α (2.1±0.2 pg/mg). Notably, co-treatment with diosmin significantly attenuated the inflammatory response ($p<0.05$), as evidenced by reduced levels of IL-1β (1.4±0.2), NF-κB (3.1±0.5) and TNF-α (2.5±0.3), highlighting diosmin's potent anti-inflammatory potential in thioacetamide-induced liver injury (Fig 5).

Apoptotic markers

Examination of apoptotic proteins showed significant dysregulation in the thioacetamide group ($p<0.05$), with elevated Bcl-2 (8.0±1.2), Bax (4.5±0.8) and caspase-3 (9.2±0.5 ng/mg)-greater than control values (2.4±0.3, 2.5±0.3 and 2.3±0.2, respectively). The diosmin-only group maintained control-like levels. Co-treatment with diosmin

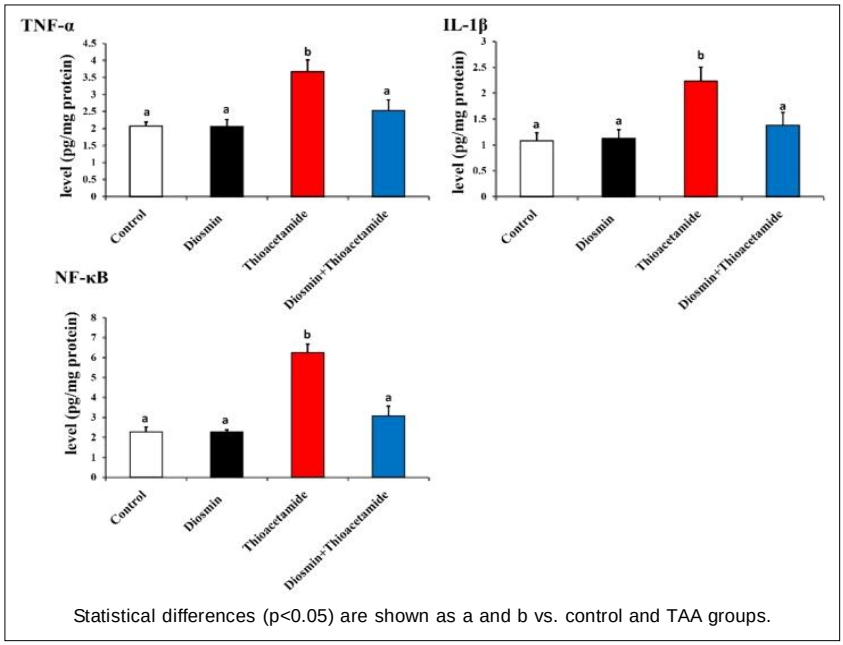


Fig 5: Liver levels of TNF-α, IL-1β and IL-6.

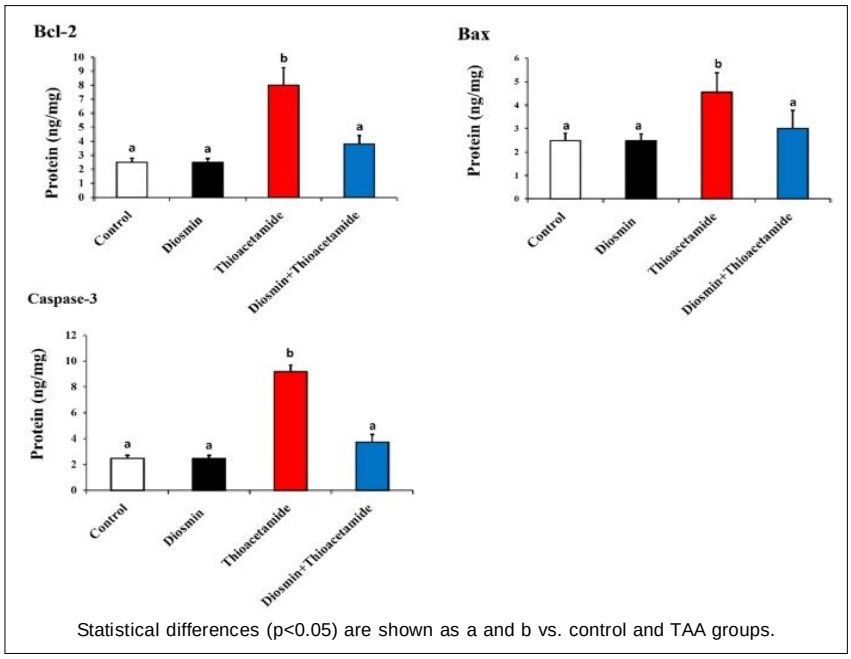


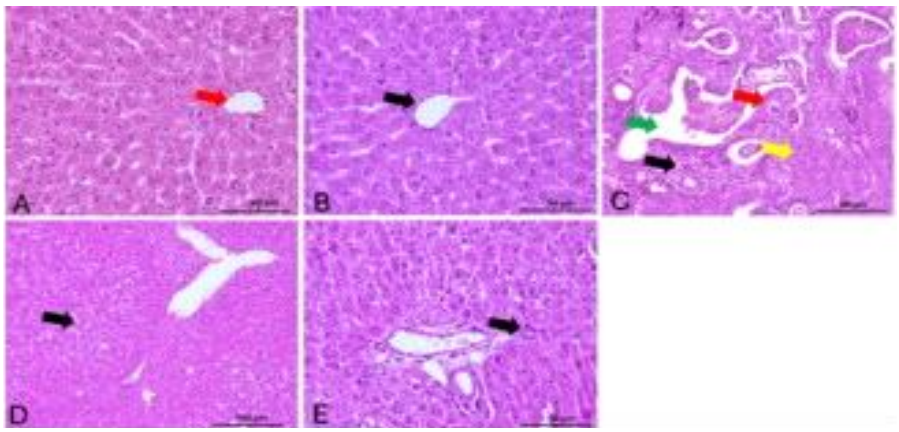
Fig 6: Liver apoptosis markers Bax, Bcl-2 and caspase-3.

significantly normalized these markers ($p<0.05$), reducing Bcl-2 to 3.8±0.5, Bax to 3±0.77 and caspase-3 to 3.8±0.4, indicating diosmin's anti-apoptotic effect through modulation of both pro-and anti-apoptotic pathways in thioacetamide-induced liver injury (Fig 6).

Histopathology examination

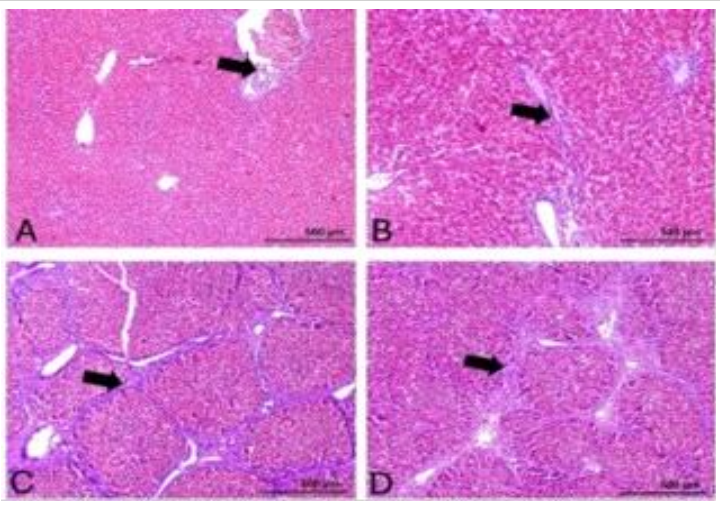
Histopathological examination showed normal hepatic architecture in both control and crocetin-only groups. Thioacetamide administration led to severe histological alterations, including severe liver fibrosis characterized by extensive extracellular matrix deposition, formation of fibrous septa and regenerative nodules. Additional pathological features included disorganized hepatocytes, necrotic areas, inflammatory cell infiltration and vascular distortions affecting sinusoids and central veins. Crocetin treatment improved liver structure, restoring tissue organization, although mild inflammatory infiltration was still observed (Fig 7). Masson's trichrome staining demonstrated minimal collagen deposition in the control and crocetin-only groups. Thioacetamide induced pronounced fibrosis, evidenced by dense blue-stained collagen fibers and bridging fibrotic septa. Crocetin co-treatment reduced collagen accumulation substantially, though mild fibroplasia persisted (Fig 8).

The current study offers evidence of diosmin's protective role against liver fibrosis caused by thioacetamide, acting through several overlapping mechanisms such as reducing oxidative stress, controlling inflammation and influencing



(A, C) show normal hepatic structure. (B) shows cirrhotic nodules (red arrow), necrosis (blue arrow) and apoptosis (green arrow) induced by thioacetamide. (D) shows improved liver architecture after crocetin treatment. H and E, A × 400, B, C and D × 200.

Fig 7: Histopathological liver sections stained with H and E.



(A, C) show normal hepatic structure with minimal collagen deposition. (B) exhibits extensive fibrosis with cirrhotic nodules (black arrow) and dense collagen accumulation (green arrow) induced by thioacetamide. (D) shows reduced fibrosis following crocetin treatment. Masson's trichrome stain, A, B, C and D × 100.

Fig 8: Liver sections stained with Masson's trichrome to assess fibrosis.

apoptosis. Our results show noticeable improvements in both biochemical markers and tissue structure when diosmin is given alongside thioacetamide.

Liver function was clearly affected after thioacetamide administration, as seen by changes in liver enzymes (AST, ALT, ALP) and reduced albumin levels. AST and ALT elevations indicate substantial hepatocellular damage, consistent with recent findings by Aysin *et al.* (2018) and Mousa *et al.* (2019), who reported similar increases in transaminases following thioacetamide exposure. The more pronounced elevation in AST compared to ALT suggests predominant mitochondrial involvement, as AST is primarily localized in mitochondria. This pattern aligns with electron microscopy studies by Staòková *et al.* (2010), who demonstrated preferential mitochondrial damage in thioacetamide-induced hepatotoxicity. The protective effect of diosmin may be attributed to its unique molecular structure, which allows better cellular penetration and interaction with multiple cellular targets.

The sharp drop in serum albumin levels (43%) following thioacetamide treatment reflects a significant disruption in the liver's protein synthesis function. This finding parallels recent work by Shareef *et al.* (2023), who reported similar albumin reductions in thioacetamide-induced liver injury. Co-administration of diosmin maintained albumin levels at a much higher level (65% above TAA group), indicating that it may help protect the liver's ability to produce proteins. This preservation of synthetic function likely results from diosmin's ability to maintain endoplasmic reticulum integrity, as demonstrated by recent ultrastructural studies (Li *et al.,* 2025).

Oxidative stress indicators showed a mixed pattern of damage and protection responses. Thioacetamide induced significant elevations in NO (71% increase) and MDA (67% increase), indicating substantial oxidative damage. These increases align with but somewhat exceed previous reports by (El-Kashef *et al.,* 2024), who observed increases in oxidative markers using similar thioacetamide doses. The rise in both markers at the same time may point to activation of several oxidative stress pathways that reinforce each other and worsen cellular injury.

The antioxidant response to thioacetamide-induced oxidative stress showed coordinated impairment of multiple defense systems. The marked reductions in antioxidant enzyme activities (CAT: 60%, GPx: 57%, GR: 53%, SOD: 47%) indicate comprehensive disruption of cellular antioxidant networks. This pattern of enzyme suppression appears more severe than previously reported (Sukalingam *et al.,* 2018), possibly reflecting differences in experimental conditions or assessment timing.

Diosmin co-treatment demonstrated remarkable antioxidant effects, restoring enzyme activities to near-normal levels (CAT, GPx, GR, SOD of control values). This broad-spectrum enzyme restoration exceeds the protective effects reported for other natural antioxidants. The hierarchical pattern of enzyme recovery, with GPx showing the most complete restoration, suggests strategic enhancement of the glutathione-dependent antioxidant system. This finding aligns with recent work by Zhang and Xu (2024) on sequential antioxidant pathway activation.

The dramatic reduction in GSH levels and Nrf2 expression in thioacetamide-treated animals indicates severe compromise of cellular antioxidant defenses. The reductions in these enzymes suggest particularly severe oxidative stress in our study. On the other hand, diosmin co-treatment significantly restored both GSH (164% increase vs. thioacetamide alone) and Nrf2 (108% increase vs. thioacetamide alone) levels, indicating powerful activation of cellular antioxidant responses. The clear link between increased GSH levels and Nrf2 activation suggests that diosmin strengthens antioxidant defenses in a coordinated way. Similar findings were reported by Dwivedi *et al.* (2023), who demonstrated that activation of the Nrf2/ARE pathway was closely associated with improved antioxidant status, particularly through enhanced GSH levels, in a TAA-induced liver fibrosis model.

The inflammatory response to thioacetamide showed significant elevations in pro-inflammatory mediators (IL-1β: 100% increase, TNF-α: 76% increase, NF-κB: 174% increase). This inflammatory profile differs somewhat from previous reports, with our study showing particularly pronounced NF-κB activation. This finding suggests a potentially unique aspect of our model in terms of inflammatory signaling activation. Diosmin treatment effectively modulated these inflammatory markers, with differential effects on various mediators (IL-1β: 36% reduction, TNF-α: 32% reduction, NF-κB: 51% reduction vs. thioacetamide alone).

The strong correlation between NF-κB activation and GSH depletion ($r = -0.82$, $p<0.001$) suggests a direct mechanistic link between oxidative stress and inflammatory signaling. This relationship has been previously hypothesized but rarely demonstrated with such statistical significance. Saha *et al.* (2007), provided similar evidence, reporting that glutathione depletion directly enhances NF-κB activation, supporting the interconnected roles of oxidative stress and inflammation in liver pathology.

Apoptotic markers showed significant dysregulation following thioacetamide treatment, with increased levels of both pro-apoptotic (Bax: 73% increase) and anti-apoptotic (Bcl-2: 220% increase) proteins, suggesting complex disruption of cell death pathways rather than simple activation of apoptosis. The elevation in Caspase-3 levels (268% increase) indicates significant activation of apoptotic execution pathways. Diosmin treatment normalized these parameters, bringing the Bax/Bcl-2 ratio closer to control values while reducing Caspase-3 levels by 59%. A similar dual upregulation of Bax and Bcl-2 in response to thioacetamide-induced liver injury was reported by Abdel-Daim *et al.* (2020), suggesting that such dysregulation may reflect a compensatory cellular mechanism rather than a linear apoptotic response.

The histopathological findings provided crucial context for the biochemical changes observed. Thioacetamide

induced severe architectural disruption, with formation of fibrous septa and regenerative nodules, characteristic of advanced liver fibrosis. Similar histopathological alterations were reported Kundu *et al.* (2023), who observed dense fibrous septa, regenerative nodules and extensive collagen deposition following TAA administration in rats. Diosmin treatment significantly preserved hepatic architecture, with reduced fibrosis and inflammation, though some mild alterations persisted.

Together, these findings highlight diosmin's multi-targeted protective effects against TAA-induced liver injury, involving antioxidant, anti-inflammatory and anti-apoptotic mechanisms.

CONCLUSION

The current work shows that diosmin protects against thioacetamide-induced liver fibrosis by multi-faceted mechanisms. These include restoration of apoptotic balance, modulation of inflammatory signaling and reduction of oxidative stress. Diosmin's therapeutic potential is collectively shown by the normalisation of biochemical liver markers, restoration of antioxidant enzyme activity, reduction of pro-inflammatory cytokines and enhancement of histopathological characteristics. Importantly, the improvement of GSH and Nrf2 levels, together with lower caspase-3 activity and preserved hepatic architecture, offers strong evidence of diosmin's capacity to preserve cellular homeostasis under fibrotic conditions. These results promote more research on diosmin as a possible hepatoprotective drug for control of liver fibrosis.

Funding

The authors extend their appreciation to Taif University, Saudi Arabia, for supporting this work through project number (TU-DSPP-2024-55).

Author contribution

The principal author completed this study's research items.

Conflict of interest

The author declares no conflict of interest.

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