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Diosmin as a Hepatoprotective Flavonoid: Mechanistic Insights into Its Antioxidant and Anti-Inflammatory Protection Against Atorvastatin-Induced Hepatotoxicity

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ABSTRACT

Background: Atorvastatin, a widely prescribed lipid-lowering agent, has been associated with idiosyncratic hepatotoxicity in susceptible individuals despite its favorable cardiovascular benefits. The hepatotoxic effects of atorvastatin are largely attributed to oxidative stress, mitochondrial dysfunction, and inflammatory cascades triggered by excessive reactive oxygen species (ROS) and cytokine release. Diosmin, a natural flavonoid glycoside predominantly found in citrus fruits, exhibits potent antioxidant, anti-inflammatory, and membrane-stabilizing properties that have attracted growing pharmacological interest. Recent experimental and preclinical findings suggest that diosmin exerts a protective influence against chemically and drug-induced hepatic damage through modulation of oxidative and inflammatory signaling pathways. This review aims to comprehensively analyze the potential protective role of diosmin against atorvastatin-induced liver injury, emphasizing mechanistic insights into its antioxidant and anti-inflammatory actions. The mechanistic discussion integrates evidence from in vivo and in vitro studies elucidating diosmin's capacity to enhance antioxidant defenses by upregulating enzymatic activities of superoxide dismutase, catalase, and glutathione peroxidase, while concurrently suppressing lipid peroxidation and malondialdehyde accumulation. Additionally, diosmin appears to modulate inflammatory responses via downregulation of pro-inflammatory mediators such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and inducible nitric oxide synthase (iNOS), alongside inhibition of nuclear factor-κB (NF-κB) activation. By integrating current findings on the pharmacodynamics, molecular targets, and histopathological outcomes, this review highlights the dual modulatory effect of diosmin on oxidative and inflammatory pathways, offering a promising pharmacological intervention against statin-associated hepatotoxicity. Understanding these interactions may contribute to developing adjuvant therapeutic strategies that minimize the hepatic adverse effects of statin therapy while preserving its cardiovascular efficacy. In conclusion, diosmin represents a potential hepatoprotective agent with multifaceted mechanisms that counteract atorvastatin-induced hepatic injury. Further translational and clinical studies are warranted to delineate optimal dosing regimens, pharmacokinetic interactions, and long-term safety profiles, establishing diosmin as a viable complementary therapy in patients requiring statin treatment.

Keywords: *Diosmin, Hepatoprotective Flavonoid, Atorvastatin-Induced hepatotoxicity*

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INTRODUCTION

Atorvastatin, one of the most prescribed statins worldwide, plays a pivotal role in the management of hypercholesterolemia and the prevention of cardiovascular events through inhibition of the hepatic enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase. Despite its clinical efficacy, evidence indicates that atorvastatin can provoke varying degrees of hepatotoxicity, ranging from mild transaminase elevation to severe hepatic injury. The underlying mechanisms are complex and multifactorial, involving oxidative stress, mitochondrial dysfunction, and inflammatory responses that collectively disrupt hepatocellular integrity and function. Such adverse hepatic outcomes can limit therapeutic adherence and necessitate discontinuation of statin therapy in certain patient populations, underscoring the need for safer co-therapeutic strategies [1].

In recent years, interest has grown in the use of natural bioflavonoids as hepatoprotective agents, owing to their potent antioxidant and anti-inflammatory properties. Diosmin, a naturally occurring flavonoid glycoside found in citrus fruits, has demonstrated broad pharmacological activities, including vascular protection, free radical scavenging, and modulation of inflammatory mediators. Pharmacologically, diosmin exhibits high bioavailability and metabolic stability, making it an appealing candidate for mitigating oxidative and inflammatory hepatic insults induced by various xenobiotics, including statins [2].

Although atorvastatin-induced hepatotoxicity has been well-documented in clinical and experimental models, the exploration of diosmin's protective mechanisms in this specific context remains limited. Current literature reveals promising evidence of diosmin's capacity to modulate oxidative stress markers, enhance antioxidant enzyme expression, and suppress inflammatory signaling pathways, yet a comprehensive understanding of its hepatoprotective efficacy against statin-induced hepatic injury is still lacking [3]. This gap in mechanistic insight forms the foundation of the present review.

The aim of this review is to provide an integrated analysis of the pharmacological mechanisms underlying diosmin's protective effects against atorvastatin-induced hepatotoxicity, emphasizing its antioxidant and anti-inflammatory pathways. By synthesizing evidence from biochemical, molecular, and histopathological studies, this review seeks to elucidate how diosmin attenuates hepatic oxidative and inflammatory damage, potentially offering a novel adjunctive therapeutic approach to enhance the hepatic safety of statin therapy [4].

Atorvastatin-Induced Hepatotoxicity: Mechanisms and Pathophysiology

Atorvastatin, a synthetic lipid-lowering agent, functions primarily by competitively inhibiting HMG-CoA reductase, thereby reducing endogenous cholesterol synthesis and increasing low-density lipoprotein (LDL) receptor expression in hepatocytes. Despite its cardiovascular benefits, clinical and experimental data consistently report instances of hepatic dysfunction, which range from asymptomatic elevations in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) to

10.48047/jocaaa.2024.33.06.143

severe hepatocellular necrosis. The hepatotoxicity is idiosyncratic in nature and is believed to involve both intrinsic biochemical toxicity and immune-mediated mechanisms [5].

Atorvastatin undergoes extensive hepatic metabolism through cytochrome P450 (CYP) 3A4 isoenzymes, generating reactive intermediates that can disrupt hepatocellular redox balance. These metabolites can covalently bind to intracellular macromolecules, triggering oxidative stress and mitochondrial injury. Such mitochondrial dysfunction leads to impaired oxidative phosphorylation, increased production of reactive oxygen species (ROS), and subsequent lipid peroxidation of cell membranes [6]. The resultant oxidative damage compromises hepatocyte viability, manifesting histopathologically as vacuolar degeneration, cytoplasmic ballooning, and focal necrosis [7].

Oxidative stress serves as a crucial initiator and amplifier of atorvastatin-induced hepatic injury. The excessive accumulation of ROS depletes endogenous antioxidants such as glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT), culminating in an imbalance between pro-oxidant and antioxidant systems. This disequilibrium not only damages lipids and proteins but also promotes DNA fragmentation, ultimately leading to apoptotic or necrotic cell death. The nuclear translocation of NF-κB and activation of mitogen-activated protein kinases (MAPKs) further propagate inflammatory cascades that exacerbate tissue damage [8].

Inflammatory cytokines play a central role in the progression of statin-induced hepatic injury. Tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6) are key mediators released in response to oxidative stress and hepatocyte injury. These cytokines amplify the inflammatory microenvironment by recruiting immune cells such as macrophages and neutrophils, which in turn generate more ROS and nitric oxide (NO), creating a vicious cycle of oxidative and inflammatory damage. Studies have demonstrated that statin-induced liver injury is associated with elevated hepatic expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2), further intensifying oxidative and inflammatory stress [9].

Beyond oxidative and inflammatory pathways, atorvastatin may also exert hepatotoxic effects by impairing mitochondrial β-oxidation and altering bile acid metabolism. The interference with mitochondrial fatty acid oxidation can lead to triglyceride accumulation and steatosis, while disruption of bile acid homeostasis may contribute to cholestatic injury. Recent studies indicate that statins can upregulate pro-apoptotic proteins such as Bax and downregulate anti-apoptotic factors like Bcl-2, tipping the balance toward programmed hepatocyte death [10].

In summary, atorvastatin-induced hepatotoxicity is a multifactorial process involving oxidative stress, mitochondrial dysfunction, inflammatory signaling, and apoptosis. The convergence of these mechanisms underscores the importance of antioxidant and anti-inflammatory interventions in mitigating hepatic injury. Diosmin, through its multifaceted pharmacological profile, emerges as a promising candidate capable of counteracting these pathological pathways [11].

10.48047/jocaaa.2024.33.06.143

Pharmacological Profile and Biological Activities of Diosmin

Diosmin (3′,5,7-trihydroxy-4′-methoxyflavone-7-rutinoside) is a naturally occurring flavonoid glycoside, primarily derived from hesperidin found in citrus fruits such as oranges and lemons. Pharmacologically, diosmin has been recognized for its broad therapeutic potential due to its antioxidant, anti-inflammatory, vasoprotective, and anticarcinogenic properties. Its extensive pharmacological activities have positioned it as a valuable bioflavonoid in the management of chronic venous insufficiency, hemorrhoidal disease, and vascular disorders, while emerging evidence also highlights its organoprotective effects against oxidative tissue injury [12].

Following oral administration, diosmin undergoes hydrolysis by intestinal microbiota into its aglycone form, diosmetin, which is readily absorbed and subsequently subjected to hepatic metabolism involving phase I and phase II reactions. Diosmetin is primarily conjugated to glucuronides and sulfates, facilitating systemic circulation and eventual biliary excretion. The bioavailability of diosmin, although moderate, is sufficient to elicit sustained pharmacological responses due to its prolonged elimination half-life and enterohepatic recycling. Notably, its pharmacokinetic behavior is characterized by extensive distribution in hepatic and vascular tissues, where it exerts its biological effects [13].

Diosmin's potent antioxidant activity stems from its polyphenolic structure, which enables direct scavenging of reactive oxygen and nitrogen species, as well as the upregulation of endogenous antioxidant defense systems. Studies have demonstrated that diosmin enhances the activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), while simultaneously elevating reduced glutathione (GSH) levels. This combined effect mitigates lipid peroxidation and preserves cellular redox homeostasis. Furthermore, diosmin suppresses oxidative damage to cellular lipids, proteins, and DNA, thereby stabilizing hepatocyte membranes and preventing apoptotic signaling induced by ROS accumulation [14].

The anti-inflammatory effects of diosmin are mediated through modulation of key inflammatory signaling pathways. Diosmin inhibits the activation of nuclear factor kappa B (NF-κB), a central regulator of pro-inflammatory gene expression. By attenuating NF-κB translocation and its downstream mediators such as tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), and inducible nitric oxide synthase (iNOS), diosmin effectively curtails the inflammatory cascade. Additionally, diosmin interferes with the cyclooxygenase-2 (COX-2) pathway, thereby reducing prostaglandin synthesis and inflammatory cell infiltration. These dual antioxidant and anti-inflammatory actions contribute synergistically to diosmin's hepatoprotective efficacy [15].

Beyond its antioxidant and anti-inflammatory effects, diosmin exhibits membrane-stabilizing and mitochondrial-protective properties. It maintains mitochondrial membrane potential, inhibits cytochrome c release, and prevents activation of caspase-dependent apoptosis. Diosmin also improves

10.48047/jocaaa.2024.33.06.143

hepatic microcirculation by enhancing nitric oxide bioavailability and reducing endothelin-1 levels, facilitating oxygen and nutrient delivery to hepatocytes. Collectively, these pharmacological attributes support diosmin's protective capacity against hepatocellular injury induced by xenobiotics, including statins [16].

In summary, diosmin is a multifaceted bioflavonoid with diverse pharmacodynamic effects relevant to hepatic protection. Its ability to regulate oxidative stress, inflammatory responses, and mitochondrial function positions it as a promising therapeutic adjunct in drug-induced hepatotoxicity, particularly in mitigating atorvastatin-related hepatic injury [17].

Experimental Evidence of Diosmin's Protective Role Against Atorvastatin-Induced Hepatic Injury

Several preclinical studies have demonstrated the hepatoprotective efficacy of diosmin against chemically and pharmacologically induced liver damage, with growing attention on its role in mitigating atorvastatin-induced hepatotoxicity. Experimental data from animal models indicate that diosmin supplementation significantly reduces hepatic enzyme leakage, oxidative stress biomarkers, and histopathological damage induced by statin exposure. In a recent rat study, atorvastatin administration caused marked elevations in serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and bilirubin levels—biochemical indicators of hepatocellular membrane disruption. Co-administration of diosmin markedly normalized these parameters, suggesting stabilization of hepatic membranes and restoration of functional integrity [18].

Histopathological analyses in atorvastatin-exposed rats revealed centrilobular necrosis, inflammatory infiltration, and hepatic vacuolization, all of which were ameliorated upon diosmin treatment. The hepatocellular architecture in diosmin-treated groups appeared near normal, with reduced cellular degeneration and minimal inflammatory cell presence. These morphological findings corroborate biochemical evidence, indicating diosmin's ability to attenuate hepatic damage through suppression of oxidative and inflammatory responses. Moreover, immunohistochemical staining showed a decline in the expression of NF-κB and caspase-3 in hepatic tissues following diosmin administration, confirming its anti-inflammatory and anti-apoptotic effects [19].

At the molecular level, diosmin enhances the antioxidant defense system by upregulating the mRNA expression and enzymatic activities of SOD, CAT, and GPx. This upregulation helps neutralize superoxide anions and hydrogen peroxide, preventing lipid peroxidation and malondialdehyde (MDA) accumulation. Diosmin also elevates hepatic glutathione (GSH) levels, thereby maintaining redox equilibrium and protecting against oxidative DNA fragmentation. In atorvastatin-induced toxicity models, diosmin administration significantly reduced MDA levels and increased antioxidant enzyme activities, restoring hepatic redox homeostasis [20].

10.48047/jocaaa.2024.33.06.143

The anti-inflammatory effect of diosmin in statin-exposed animals is mediated through the suppression of key pro-inflammatory mediators. Diosmin downregulates TNF-α, IL-1β, and IL-6 expression, thereby preventing cytokine-induced hepatocyte injury. In addition, diosmin inhibits the nuclear translocation of NF-κB and reduces the activation of inducible nitric oxide synthase (iNOS), leading to decreased nitric oxide and peroxynitrite formation. These findings suggest that diosmin exerts hepatoprotection not only by neutralizing free radicals but also by interrupting inflammatory signaling cascades responsible for amplifying tissue injury [21].

Furthermore, diosmin's ability to protect mitochondrial function represents an additional mechanism of hepatoprotection. Atorvastatin has been reported to induce mitochondrial dysfunction, characterized by loss of membrane potential, ATP depletion, and cytochrome c release. Diosmin counteracts these effects by stabilizing the mitochondrial membrane potential and preventing the release of apoptogenic factors. Experimental evidence shows diosmin-treated hepatocytes maintain mitochondrial integrity, accompanied by decreased expression of pro-apoptotic proteins such as Bax and increased levels of anti-apoptotic Bcl-2, thus preventing caspase-mediated apoptosis [22].

Collectively, these experimental findings highlight diosmin's multifactorial protective role against atorvastatin-induced hepatic injury. Through modulation of oxidative, inflammatory, and apoptotic pathways, diosmin restores hepatocellular homeostasis and structural integrity, reinforcing its potential as a therapeutic adjunct in statin therapy to minimize hepatotoxic risk [23].

Mechanistic Insights – Diosmin's Modulation of Oxidative Stress Pathways in Atorvastatin-Induced Hepatotoxicity

Oxidative stress represents a central mechanism in atorvastatin-induced hepatotoxicity, primarily through the generation of reactive oxygen species (ROS) that damage lipids, proteins, and DNA. Diosmin, with its polyhydroxylated flavone structure, exerts direct and indirect antioxidant effects that neutralize these deleterious radicals. Its phenolic hydroxyl groups enable hydrogen donation to reactive radicals, terminating chain reactions of lipid peroxidation. In parallel, diosmin enhances endogenous antioxidant systems by inducing transcriptional activation of antioxidant response elements (AREs) through nuclear factor erythroid 2-related factor 2 (Nrf2) signaling. Upon diosmin treatment, Nrf2 dissociates from its cytoplasmic inhibitor, Kelch-like ECH-associated protein 1 (Keap1), and translocates into the nucleus, where it promotes the expression of antioxidant genes such as heme oxygenase-1 (HO-1), NAD(P)H: quinone oxidoreductase-1 (NQO1), and glutamate–cysteine ligase (GCL) [24].

Through activation of Nrf2-dependent transcription, diosmin restores the oxidative balance in hepatocytes subjected to atorvastatin toxicity. This protective response reduces lipid peroxidation and malondialdehyde (MDA) accumulation, while simultaneously increasing glutathione (GSH) synthesis and enhancing the activities of superoxide dismutase (SOD), catalase (CAT), and glutathione

10.48047/jocaaa.2024.33.06.143

peroxidase (GPx). Studies have demonstrated that diosmin supplementation in atorvastatin-exposed rats significantly elevated hepatic GSH content and SOD activity, accompanied by decreased levels of ROS and MDA. These findings highlight the role of diosmin as both a free radical scavenger and a transcriptional activator of antioxidant defenses [25].

The ability of diosmin to attenuate oxidative damage extends to its modulation of mitochondrial oxidative pathways. Atorvastatin-induced hepatotoxicity often involves mitochondrial respiratory chain impairment, leading to excessive superoxide anion formation and secondary hydrogen peroxide accumulation. Diosmin protects mitochondria by preserving the integrity of complex I and III activities, reducing electron leakage and subsequent ROS generation. Furthermore, diosmin prevents mitochondrial depolarization and maintains adenosine triphosphate (ATP) levels, thereby sustaining cellular energy metabolism. These mitochondrial-protective effects are closely associated with diosmin's capacity to maintain reduced glutathione pools within mitochondria, which act as a first line of defense against ROS [26].

Another critical aspect of diosmin's antioxidant mechanism involves suppression of lipid peroxidation. The liver, being rich in polyunsaturated fatty acids, is particularly vulnerable to peroxidative injury caused by ROS. Diosmin inhibits peroxidative chain reactions by scavenging peroxyl radicals and reducing the formation of conjugated dienes and thiobarbituric acid-reactive substances (TBARS). The consequent reduction in lipid peroxidation preserves hepatocellular membrane fluidity and prevents leakage of cytosolic enzymes such as ALT and AST. This preservation of membrane stability contributes significantly to diosmin's hepatoprotective effect [27].

Beyond the direct antioxidant activity, diosmin modulates redox-sensitive signaling pathways that orchestrate cellular responses to oxidative stress. It downregulates the expression of NADPH oxidase (NOX) subunits, such as gp91^phox and p47^phox, which are major enzymatic sources of ROS in hepatocytes. By inhibiting NOX activation, diosmin suppresses the upstream generation of ROS, reducing oxidative injury at its source. Simultaneously, diosmin interferes with the crosstalk between ROS and NF-κB signaling, limiting oxidative-stress-induced inflammation. Through these integrated mechanisms, diosmin orchestrates a comprehensive protective network that shields hepatocytes from oxidative injury associated with atorvastatin metabolism [28].

Taken together, diosmin's capacity to modulate oxidative stress involves a multifaceted mechanism encompassing direct free radical scavenging, enhancement of enzymatic antioxidant defenses, activation of Nrf2/ARE signaling, suppression of lipid peroxidation, and inhibition of ROS-generating enzymes. These cumulative actions not only restore redox homeostasis but also prevent the subsequent inflammatory and apoptotic cascades that underlie atorvastatin-induced liver injury [29].

10.48047/jocaaa.2024.33.06.143

Anti-Inflammatory Mechanisms of Diosmin in Atorvastatin-Induced Liver Injury

The inflammatory component of atorvastatin-induced hepatic injury is tightly linked to oxidative stress–driven activation of pattern-recognition receptors and redox-sensitive transcription factors. Diosmin interrupts this feed-forward loop at multiple nodes, beginning with the suppression of early cytokine surges. In vivo models demonstrate that diosmin blunts hepatic and systemic spikes of TNF-α, IL-1β, and IL-6 following statin exposure, thereby reducing downstream endothelial activation, leukocyte recruitment, and sinusoidal congestion that perpetuate parenchymal damage. By curtailing these proximal signals, diosmin attenuates the chemotactic gradient that otherwise sustains inflammatory cell trafficking into the liver lobule. [30]

Central to diosmin's anti-inflammatory action is inhibition of NF-κB signaling. At baseline, NF-κB is retained in the cytoplasm by IκBα; oxidative stress and toll-like receptor (TLR) activation trigger IKK-mediated IκBα phosphorylation and degradation, enabling p65/p50 nuclear translocation. Diosmin stabilizes IκBα, decreases IKK phosphorylation, and limits p65 nuclear accumulation, resulting in reduced transcription of canonical NF-κB target genes encoding TNF-α, IL-1β, IL-6, COX-2, and iNOS. Functionally, this translates into lower nitric oxide and prostaglandin E2 output, decreased sinusoidal endothelial swelling, and diminished parenchymal ballooning after atorvastatin challenge. [31]

Diosmin also modulates inflammasome activity, particularly the NLRP3 complex that converts pro-caspase-1 to active caspase-1, promoting maturation of IL-1β and IL-18. Atorvastatin-evoked mitochondrial ROS, oxidized mitochondrial DNA, and potassium efflux serve as NLRP3 triggers; diosmin's mitochondrial-protective and ROS-scavenging effects dampen this activation threshold. Experimental data show reduced hepatic NLRP3, ASC, and cleaved caspase-1 levels with diosmin co-treatment, accompanied by lower IL-1β bioactivity and decreased pyroptotic signaling—key steps in halting necroinflammatory propagation across the lobular plate. [32]

At the eicosanoid axis, diosmin downregulates COX-2 and 5-lipoxygenase (5-LOX) expression, rebalancing prostanoid/leukotriene production toward a less pro-inflammatory milieu. This shift lowers PGE2-mediated vasodilation and vascular permeability while limiting leukotriene B4–driven neutrophil adhesion and transmigration. In parallel, diosmin reduces iNOS induction and nitrosative stress, curbing peroxynitrite formation that otherwise nitrates mitochondrial and cytoskeletal proteins, amplifies hepatocyte dysfunction, and sustains DAMP-mediated immune activation after statin exposure. [33]

Macrophage polarization constitutes another critical target. Kupffer cells and recruited monocyte-derived macrophages adopt an M1-dominant phenotype under statin-induced oxidative stress, secreting TNF-α, IL-1β, and reactive nitrogen species. Diosmin skews hepatic macrophage programming toward an M2-repair phenotype, evidenced by higher Arg1 and CD206 expression and

10.48047/jocaaa.2024.33.06.143

dampened iNOS and CD86 signatures. This immunometabolic reprogramming reduces cytotoxic effector functions, enhances efferocytosis of apoptotic bodies, and promotes resolution pathways (e.g., TGF-β–mediated matrix remodeling) without tipping into fibrogenic activation. [34]

Finally, diosmin restrains stress-activated kinases that bridge inflammation with cell death. By limiting upstream ROS/TLR signals, diosmin reduces phosphorylation of JNK and p38 MAPK, thereby decreasing c-Jun–dependent transcription of pro-inflammatory genes and interrupting JNK-Bax crosstalk that primes mitochondria for apoptosis. Concurrent attenuation of ERK-driven COX-2 induction complements NF-κB inhibition, yielding a coherent, pathway-spanning anti-inflammatory profile that integrates with its antioxidant and anti-apoptotic actions to blunt the full spectrum of atorvastatin-induced hepatic injury. [35]

Anti-Apoptotic and Cytoprotective Cascades (Bcl-2 Family Balance, Caspase Axis, and Mitochondrial Checkpoints)

Atorvastatin-induced hepatotoxicity commonly converges on the intrinsic apoptosis pathway, where mitochondrial outer membrane permeabilization (MOMP) represents the point of no return. Oxidative and inflammatory cues tilt the Bcl-2 rheostat toward death by upregulating Bax/Bak and suppressing anti-apoptotic Bcl-2/Bcl-xL, facilitating cytochrome c escape and apoptosome assembly. Diosmin counterbalances this shift by restoring Bcl-2 and Bcl-xL expression while repressing Bax translocation to mitochondria, thereby stabilizing the mitochondrial membrane and preventing MOMP. Functionally, this translates into lower cytosolic cytochrome c and diminished caspase-9/3 activation in statin-challenged livers, aligning its antioxidant and anti-inflammatory effects with genuine mitochondrial checkpoint control. [36–38]

Caspase cascade containment is a second, tightly linked node of diosmin's protection. By limiting upstream MOMP and suppressing stress kinases (notably JNK) that prime the Bax conformational change, diosmin reduces initiator caspase-9 activation and the downstream executioner caspase-3 cleavage responsible for chromatin condensation and DNA fragmentation. Complementary inhibition of extrinsic inputs—through decreased TNF-α/TNFR1 signaling and curtailed DISC formation—further lowers caspase-8–driven crosstalk into the mitochondrial axis via Bid truncation. These cooperative effects produce coherent reductions in TUNEL positivity and serum cytokeratin-18 fragments, indicating meaningful preservation of hepatocyte viability despite atorvastatin exposure. [39,40]

Mitochondrial permeability transition pore (mPTP) regulation and bioenergetic rescue add depth to diosmin's cytoprotection. In statin toxicity, calcium overload and ROS open the mPTP, collapsing ΔΨm, depleting ATP, and amplifying necro-apoptotic signaling. Diosmin curbs Ca2+ dysregulation, preserves ΔΨm, and supports ATP synthesis—mechanisms that are reinforced by improved antioxidant buffering (GSH, SOD, GPx) within the mitochondrial matrix. Evidence of reduced cardiolipin

10.48047/jocaaa.2024.33.06.143

peroxidation and maintained respiratory complex I/III activities under diosmin co-treatment underscores a direct mitochondrial stabilizing effect that prevents the bioenergetic crisis driving cell death. [36,41]

Endoplasmic reticulum (ER) stress is an upstream amplifier of mitochondrial apoptosis in statin-injured livers via PERK–eIF2α–ATF4 and CHOP induction. Diosmin attenuates maladaptive unfolded protein response signaling, lowering CHOP and GRP78/BiP overexpression and thereby interrupting ER-to-mitochondria "death talk" through reduced ERO1α/oxidative flux and calcium efflux into mitochondria. In parallel, diosmin promotes adaptive autophagic flux (normalized LC3-II/LC3-I ratio and p62 turnover), enabling removal of damaged mitochondria (mitophagy) and limiting propagation of mitochondrial damage signals—a critical distinction between pro-survival quality control and pro-death autophagy blockade seen with severe statin injury. [37,42]

Cytoprotective kinase signaling integrates these layers. Diosmin activates PI3K/Akt and AMPK/SIRT1 axes, which phosphorylate and inhibit pro-apoptotic Bad, enhance FOXO-guided antioxidant programs, and promote PGC-1α–dependent mitochondrial biogenesis. Concurrent Nrf2/HO-1 upregulation supplies additional redox buffering and anti-apoptotic tone, while induction of heat-shock proteins (e.g., HSP70) safeguards protein folding and inhibits apoptosome assembly. Together, these networks convert diosmin's radical-scavenging capacity into durable survival signaling, explaining its ability to reduce hepatocyte loss and improve functional biomarkers during atorvastatin challenge without compromising lipid-lowering efficacy. [38,41,43]

Pharmacokinetic and Pharmacodynamic Considerations—Diosmin–Atorvastatin Interactions (Absorption, Metabolism, Transporters, and Dosing Implications)

Diosmin is hydrolyzed by intestinal microbiota to the aglycone diosmetin, which is absorbed and undergoes extensive phase-II conjugation (primarily UGT1A3/UGT1A9/UGT2B7), with biliary excretion and notable enterohepatic recycling; by contrast, atorvastatin is a high–first-pass substrate of CYP3A4 and hepatic uptake transporter OATP1B1, with efflux via BCRP and P-glycoprotein. These orthogonal pathways create plausible interaction nodes at CYP3A4, UGTs, and transporters, particularly in the gut–liver axis where concentrations of parent flavonoid and conjugates can be high. Understanding this map is essential to anticipate exposure changes and tailor monitoring when combining diosmin with atorvastatin. [44]

In vitro work with flavonoids indicates that diosmetin exerts weak-to-moderate reversible inhibition of CYP3A4 at micromolar concentrations, while diosmin (glycoside) shows minimal direct CYP inhibition; clinically, standard oral doses used for venolymphatic disease seldom reach systemic levels that produce meaningful CYP3A4 blockade, but localized intestinal inhibition (pre-systemic) remains possible. Given that atorvastatin's oral bioavailability is strongly conditioned by intestinal and hepatic

10.48047/jocaaa.2024.33.06.143

CYP3A4, even modest luminal inhibition could increase initial portal exposure; hence, caution is warranted in patients on high atorvastatin doses or those with additional CYP3A4 inhibitors. [45]

Transporter-mediated interactions may be more consequential. Several flavonoids, including diosmetin and some glucuronides, inhibit BCRP and OATP1B1 in vitro, potentially increasing atorvastatin systemic exposure by reducing efflux (BCRP) and altering hepatocyte uptake saturation kinetics (OATP1B1). While definitive clinical interaction studies with diosmin are lacking, pharmacologic plausibility and case experience with other flavonoids (e.g., grapefruit constituents) justify pragmatic safeguards: avoid mega-doses of non-standard supplements, stagger administration, and intensify early biochemical monitoring when initiating combined therapy. [46]

From a pharmacodynamic perspective, diosmin's anti-inflammatory and antioxidant effects (NF-κB and NLRP3 downregulation; Nrf2/ARE activation) are unlikely to blunt atorvastatin's LDL-lowering efficacy because HMG-CoA reductase inhibition and hepatic LDL receptor upregulation are upstream of these pathways. Instead, diosmin may complement statin therapy by lowering hepatic oxidative tone and cytokine signaling, potentially improving hepatic tolerance without altering lipid targets—though confirmation requires controlled clinical trials focused on safety endpoints (ALT/AST trajectories) rather than lipid panels alone. [47]

Dose selection should balance efficacy with interaction risk. Preclinical hepatoprotection typically used 50–100 mg/kg diosmin (rat), mapping to human-equivalent doses near 500–1,000 mg/day; in clinical practice, micronized purified flavonoid fraction (MPFF; ~90% diosmin + 10% hesperidin) is commonly administered at 1,000 mg/day, which enhances bioavailability relative to non-micronized forms. Practical strategy: take atorvastatin in the evening and schedule diosmin at a different time window (e.g., morning) to reduce peak intestinal co-exposure; avoid co-administration with potent CYP3A4 or BCRP modulators. [48]

Interindividual susceptibility matters. Patients carrying SLCO1B1*5 (c.521T>C) have higher statin exposure and toxicity risk; ABCG2 c.421C>A and ABCB1 variants can further modulate statin pharmacokinetics. Diosmin does not "correct" transporter genotypes, but by attenuating oxidative and inflammatory injury thresholds, it could raise the margin to toxicity; still, genotype-positive patients should use the lowest effective atorvastatin dose, consider alternative statins with less CYP3A4 dependence, and undertake closer laboratory follow-up when adding diosmin. [49]

A pragmatic monitoring algorithm can reduce uncertainty: (1) establish baseline ALT/AST, ALP, bilirubin, CK, and lipid profile; (2) introduce diosmin at 500–1,000 mg/day with dose staggering; (3) recheck liver enzymes at 2–4 weeks and after any atorvastatin dose change; (4) educate on myalgia, dark urine, jaundice, or pruritus; (5) if ALT/AST rise to >3× ULN or symptoms occur, pause diosmin and consider statin dose reduction or switch, then rechallenge methodically once labs normalize. This

10.48047/jocaaa.2024.33.06.143

approach aligns with hepatology safety frameworks while accommodating the mechanistic promise of diosmin. [50]

Preclinical-to-Clinical Translation—Evidence Gaps, Trial Design Considerations, and Safety Signals

Despite robust preclinical evidence, clinical data supporting diosmin's hepatoprotective role against atorvastatin-induced injury remain scarce. Most studies to date are limited to experimental rodent models or in vitro systems, which, while mechanistically informative, cannot fully replicate the complex pharmacokinetic and immunologic dynamics in humans. Translational extrapolation requires careful dose normalization and bioavailability considerations, especially since diosmin's intestinal metabolism and conjugation differ significantly between species. Therefore, well-designed clinical trials are essential to validate diosmin's safety and efficacy as an adjunct therapy for patients on long-term statin regimens [51].

Preclinical results provide a strong foundation for clinical exploration. The consistent reduction in transaminases, improved antioxidant enzyme activities, and restored hepatic histology in animal studies suggest diosmin's therapeutic potential. Pilot human studies could employ a randomized, double-blind, placebo-controlled design in dyslipidemic patients on atorvastatin, measuring liver function biomarkers (ALT, AST, ALP, bilirubin) alongside oxidative stress markers (MDA, GSH, SOD) to confirm translational efficacy. Secondary endpoints should include assessment of lipid control to ensure diosmin does not compromise atorvastatin's lipid-lowering action. Such structured evaluation would establish the clinical relevance of diosmin's hepatoprotective mechanisms [52].

Safety profiling remains favorable. Diosmin and its micronized formulations are well-tolerated even with prolonged administration, with minimal gastrointestinal side effects and negligible hepatotoxic signals. Nonetheless, potential pharmacokinetic interactions via CYP3A4 or OATP1B1 inhibition warrant monitoring in high-dose or polypharmacy scenarios. Importantly, diosmin's lack of interference with atorvastatin's cholesterol-lowering effect makes it a viable candidate for combination therapy aimed at reducing hepatic risk without therapeutic compromise [53].

Bridging preclinical to clinical translation thus requires a phased research strategy—standardization of diosmin preparations, dose-finding studies, and mechanistic biomarker validation—to establish evidence-based guidelines. With these steps, diosmin could emerge as a clinically relevant hepatoprotective adjuvant in statin-treated populations [54].

A. Conclusion

Atorvastatin remains a cornerstone in dyslipidemia management but carries an inherent risk of hepatotoxicity driven by oxidative, inflammatory, and apoptotic pathways. Diosmin, as a bioactive flavonoid, offers multifaceted hepatoprotection through its potent antioxidant, anti-inflammatory, and cytoprotective mechanisms. By enhancing endogenous antioxidant defenses, suppressing NF-κB and

10.48047/jocaaa.2024.33.06.143

NLRP3 signaling, and stabilizing mitochondrial and cellular integrity, diosmin effectively interrupts the cascade of hepatic injury initiated by statin exposure.

The cumulative experimental evidence supports diosmin's role as a promising adjunctive agent capable of mitigating atorvastatin-induced liver damage without compromising its lipid-lowering efficacy. Its ability to balance redox homeostasis, inhibit inflammatory mediators, and prevent hepatocyte apoptosis underscores its pharmacological versatility and translational potential. Furthermore, diosmin's favorable safety profile and extensive clinical experience in vascular disorders provide an encouraging foundation for therapeutic repurposing.

However, moving from bench to bedside necessitates rigorous clinical validation to determine optimal dosing, pharmacokinetic compatibility, and long-term safety within statin-treated populations. Well-structured clinical trials incorporating mechanistic biomarkers will be crucial to substantiate diosmin's hepatoprotective effects and guide its integration into statin therapy.

In conclusion, diosmin represents a compelling pharmacological strategy for enhancing hepatic safety in patients receiving atorvastatin. Its integration into clinical practice could mark a significant advancement in the prevention of statin-associated hepatotoxicity, merging natural product pharmacology with modern cardiovascular therapeutics to promote safer, more sustainable lipid management.

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