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ORIGINAL ARTICLE

Potential Protective Antioxidant and Anti-inflammatory Effects of Diosmin Against Atorvastatin-Induced Hepatotoxicity in Male Albino Rats

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ABSTRACT

Background: Atorvastatin is one of the statins that commonly cause liver injury as an adverse effect. Diosmin is one of the safest drugs. This work aimed to study the potential protective antioxidant as well as anti-inflammatory impacts of diosmin against atorvastatin-induced hepatotoxicity among the male albino rats.

Methods: Five groups of male albino rats (seven rats each) were encompassed in the study. The control group had the vehicle (2.5% DMSO, 5 ml/kg) orally for 30 days, while the atorvastatin group was given atorvastatin (40 mg/kg/day) liquified in 2.5% DMSO by oral gavage for the same duration. Two groups were co-administered atorvastatin (40 mg/kg/day) along with diosmin at doses of 100 and 200 mg/kg/day, given orally for a duration of 30 days. Another group received diosmin alone (100 mg/kg/day) for the same period. Following completion of the treatment protocol, evaluations included serum alanine aminotransferase (ALT), hepatic concentrations of tumor necrosis factor-alpha (TNF-α), malondialdehyde (MDA), nuclear factor erythroid 2–related factor 2 (Nrf2), in addition to the heme oxygenase-1 (HO-1). Additionally, liver specimens were subjected to histological assessment using hematoxylin and eosin (H&E) staining.

Results: Atorvastatin (40 mg/kg) markedly reduced weight gain (10.0 ± 0.500 g vs. 18.8 ± 0.583 g in controls, $p<0.05$), increased ALT (253.436 ±2.534 vs. 46.188 ±1.284 U/L), hepatic MDA (7.19 ± 0.237vs. 1.09 ± 0.102 nmol/mg) and TNF-α (378.95 ± 4.271vs. 116.23 ± 2.120 pg/mg), while decreasing Nrf2 (2.54 ± 0.271 vs. 19.80 ± 0.214 ng/mg protein) and HO-1 (3.05 ± 0.171vs. 16.07 ± 0.195 ng/mg protein). Diosmin co-treatment dose-dependently restored biochemical markers, with 200 mg/kg showing near-normal values, and histopathology confirming marked hepatic protection compared to atorvastatin alone.

Conclusion: Diosmin provides dose-dependent hepatoprotection against atorvastatin-induced injury, through its antioxidant in addition to its anti-inflammatory actions, highlighting the therapeutic potential for mitigating drug-induced hepatic damage.

Keywords: Diosmin; atorvastatin; liver injury; Nrf2; HO-1.

INTRODUCTION

Drug-induced liver injury (DILI) refers to ability of certain drugs, chemicals, or medical herbs to damage the liver cells. The liver serves as the central organ for metabolism of xenobiotics, a role that renders it particularly vulnerable to drug-induced toxicity and adverse reactions resulting from metabolic byproducts [1]. There are some features that increase the risk of DILI such as: gender, age, comorbidity, immune response, liver regeneration, and transporters. In addition, the lipophilicity of the drug because this might affect the hepatocyte drug absorption and reactive metabolite buildup

[2]. According to the LiverTox website, there are about 1000 drugs and herbal substances that may cause liver cell injury; one of these drugs is atorvastatin [3]. Atorvastatin does its action through inhibition of enzyme of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, thereby suppressing cholesterol formation and enhancing the clearance of low-density lipoprotein (LDL) from blood circulation, making it a widely used agent in the management of hyperlipidemia [4]. In Egypt, a study conducted at the National Liver Institute, Menoufia University, reported that DILI accounted for 1.38% of hospitalized patients over the course of one year [5].

The exact mechanism underlying statin-induced liver injury remains unclear; however, several potential pathways and explanations have been proposed to account for its occurrence [6]. Oxidative stress is a critical pathological process in DILI and other liver diseases. Statins decrease the respiratory capacity of respiratory chain complexes I, II, as well as III, resulting in superoxide ($O2^-$) generation in large quantities leading to oxidative stress [7]. Mevalonate depletion is a result of inhibition of enzyme of HMG-COA reductase resulting in a reduction of isoprenoids and isoprenoid compounds [8], and consequently, depletion of serum ubiquinone (CoQ10) that leads to production of free radicals, and mitochondrial dysfunction [9]. Oxidative stress triggers the activation of nuclear factor erythroid 2 (Nrf2), a pivotal transcription regulator responsible for inducing antioxidant gene expression and restoring the balance between pro-oxidant and antioxidant systems within the cell [10]. Oxidative stress induces hepatic inflammation thereby increasing the expression of inflammatory mediators such as TNF-α that was reported in other hepatoxic models [11,12].

Diosmin is a naturally derived flavonoid glycoside, classified as a polyphenolic compound, that is primarily present in citrus species and, to a lesser extent, in olive leaves [13]. Diosmin is prescribed for hemorrhoids and venous ulcer cases; in addition, it can prevent postoperative thromboembolism [14]. In the chemical structure of diosmin, there is a free-radical scavenger, and consequently, diosmin is considered an important antioxidant drug [15]. Diosmin is a promising drug that could provide huge potential in the treatment of hepatic diseases. It was found that diosmin can decrease the hepatic injury induced by ethanol and aflatoxin [16].

Although atorvastatin is an effective lipid-lowering agent, its clinical utility is hindered by hepatotoxic side effects that remain poorly understood at the molecular level. Current therapeutic strategies to counteract these adverse effects are limited, and safe pharmacological protectants are lacking. While diosmin has demonstrated antioxidant and anti-inflammatory potential in various models of organ injury, its protective efficacy against statin-induced hepatotoxicity has not been comprehensively investigated. Furthermore, the mechanistic involvement of pathways such as Nrf2/HO-1 signaling in this context has yet to be clearly delineated. Therefore, this work aimed to study the potential protective antioxidant in addition to the anti-inflammatory impacts of diosmin against atorvastatin-induced hepatotoxicity among the male albino rats.

METHODS

Drugs and chemicals: The study materials included atorvastatin (powder form), diosmin (powder form), and thiopental (injectable solution), the compounds were purchased from Sigma-Aldrich (St. Louis, MO, USA), and dimethyl sulfoxide (2.5%) was sourced separately from Glentham Life Science, United Kingdom.

Animals: A total of thirty-five healthy adult male albino rats, weighing between 200 and 220 grams, were obtained from the Animal Research Unit, Faculty of Veterinary Medicine, Zagazig University, Egypt. The entire experimental procedure was carried out within the same place under strict compliance with the National Institutes of Health (NIH) guidelines for the care and use of laboratory animals (NIH Publication No. 8023, revised 1978). Animals

were housed in a well-ventilated environment with regulated temperature (22 ± 3 °C) and a 12-hour light/dark cycle to minimize stress-related variability. They had ad libitum access to standard rodent chow and fresh water throughout the study. To ensure proper adaptation to laboratory conditions and to reduce experimental bias, all animals underwent a one-week acclimatization period before the start of treatments. The study protocol was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Zagazig University (Approval No. ZU-IACUC/3/F/170/2023), confirming compliance with ethical standards for animal experimentation.

Experimental design: A total of thirty-five male albino rats were randomly assigned to five distinct experimental groups to ensure unbiased allocation and comparability across treatments. Each group contained seven rats (n=7): Group 1: the control (non-treated) group. Rats in this group were given the vehicle 2.5% DMSO 5 ml/kg by oral gavage for 30 days [17]. Group 2: the atorvastatin-treated group (Ator-treated group): rats in this group received atorvastatin daily oral dose of 40 mg/kg dissolved in 2.5% DMSO for 30 days [18]. Group 3: the atorvastatin-diosmin100-treated group (Ator-Dio100-treated group): rats were given both; atorvastatin a daily oral dose of 40 mg/kg [18] and diosmin a daily oral dose of 100 mg/kg; both drugs were dissolved in 2.5%DMSO and given for 30 days [18,19]. Group 4: the atorvastatin-diosmin200-treated group (Ator-Dio200-treated group): rats were given atorvastatin a daily oral dose of 40 mg/kg [18] plus diosmin a daily oral dose of 200 mg/kg [19]; both drugs were dissolved in 2.5% DMSO for 30 days. Each drug is administrated in a separate syringe with volume of 1cm of each drug for each rat. Group 5 (Dio100-treated group): This group was included to rule out any potential hepatotoxic effect of diosmin itself. These rats received diosmin a daily oral dose of 100 mg/kg, prepared in 2.5% DMSO, for 30 consecutive days [19].

Initial and terminal body weights were documented for all animals, and weight gain was computed by evaluating the change between these two measurements.

After completion of the experiment: euthanasia was performed using thiopental sodium (40 mg/kg, i.p.) [20]. Retro-orbital blood samples were collected in plain tubes, allowed to clot for 10 minutes, and centrifuged (3000 rpm, 30 min). The resulting non-hemolyzed serum was aliquoted and frozen at –20°C until biochemical evaluation. Livers were excised, washed with isotonic saline, and processed accordingly: right lobes fixed in 10% neutral buffered formalin for histopathological examination, while the remaining tissue was snap-frozen in liquid nitrogen and preserved at –80°C for homogenate preparation.

Assay of the serum level of alanine transaminase (ALT): Serum samples were analyzed for alanine aminotransferase (ALT) activity using a kinetic enzymatic assay with commercially available kits supplied by SPINREACT, S.A./S.A.U., Ctra. Santa Coloma, Spain.

Liver homogenate preparation: Each 100 mg portion of liver tissue was homogenized in 1 ml of chilled phosphate-buffered saline (PBS, pH 7.4) and maintained at –20°C overnight. To enhance membrane rupture, homogenates underwent two freeze–thaw cycles prior to centrifugation at 3000 × g for 10 minutes at 4°C. The resulting supernatant was immediately harvested and applied to the determination of targeted biochemical parameters [21].

Assay of the hepatic levels of malondialdehyde (MDA) as well as the tumor necrosis factor (TNF-α): Malondialdehyde (MDA) was assesed by a colorimetric assay kit (TBA method) obtained from Elabscience Biotechnology WH, China. according to the manufacturers' instructions. Quantification of hepatic TNF-α was achieved via sandwich ELISA methodology using a commercially available kit from Cusabio Innovation Centre

(Houston, TX, USA), with assay steps executed as recommended by the manufacturer.

Assay of the hepatic levels of nuclear factor erythroid-related factor 2 (Nrf-2) and heme oxygenase-1 (HO-1): Nrf2 expression in liver tissues was quantified by means of a sandwich-based quantitative enzyme immunoassay utilizing a rat ELISA kit purchased from MyBioSource, USA. The same approach was applied to measure HO-1, with detection performed using a rat-specific HO-1 ELISA kit from MyBioSource, USA, strictly adhering to the procedures described by the supplier.

Histopathological examination: After fixation in formalin, liver tissues were embedded in paraffin wax, cut into sections of 4 µm thickness, and sequentially deparaffinized and rehydrated using graded ethanol solutions (100%, 90%, 70%). The sections were stained with hematoxylin and eosin (H&E) for histological examination [22]. Evaluation of hepatic architecture and injury was performed microscopically by an independent pathologist blinded to the study groups.

Statistical analysis

GraphPad Prism software (version 10) was employed for statistical analysis. The results are expressed as mean values with their standard error (SEM). Group differences were analyzed by one-way ANOVA, after which post hoc tests (Tukey's Honestly Significant Difference (HSD)) were applied where appropriate [23]. A p-value < 0.05 was regarded as statistically significant

RESULTS

I. The influence of oral diosmin (100 and 200 mg/kg/day) on body weight gain in male albino rats with liver injury induced by atorvastatin administered orally at 40 mg/kg/day. Results are reported as mean ± SEM (Figure 1):

Administration of atorvastatin was associated with a notable suppression of body weight gain when compared with the untreated control animals ($p < 0.05$). However, supplementation with diosmin—administered orally at 100 and 200 mg/kg for 30 days—produced a significant improvement in body weight gain compared with the atorvastatin-only group ($p < 0.05$). Diosmin alone with daily dose of 100mg/kg produced insignificant ($p > 0.05$) change regarding the body weight gain in relation to the normal control.

II. The impact of diosmin, administered orally at daily doses of 100 and 200 mg/kg/day for thirty consecutive days, was investigated on serum ALT levels and hepatic TNF-α in male albino rats subjected to hepatotoxicity, induced by atorvastatin (40 mg/kg/day orally) for the same duration. The outcomes were expressed as mean ± SEM (table 1):

Rats receiving atorvastatin exhibited a marked rise in serum ALT activity and hepatic TNF-α levels compared with the control group ($p < 0.05$). In contrast, concurrent administration of diosmin at daily doses of 100 and 200 milligrams per kilogram significantly lowered ALT and TNF-α levels values relative to animals treated with atorvastatin alone ($p < 0.05$). Furthermore, diosmin alone at one hundred milligrams per kilogram produced insignificant ($p > 0.05$) change regarding ALT levels meanwhile, diosmin alone reduced TNF-α levels relative to controls ($p < 0.05$)."

III. The influence of oral diosmin (100 and 200 mg/kg/day) for thirty consecutive days, was examined on hepatic MDA in male albino rats with atorvastatin-induced liver injury, administrated at daily oral dose of (40 mg/kg) for the same period. Data are presented as mean ± SEM (figure 2):

Administration of atorvastatin at forty milligrams per kilogram per day significantly elevated hepatic MDA compared with the control group ($p < 0.05$). Co-treatment with diosmin at either one hundred or two hundred milligrams per kilogram effectively mitigated these increases ($p < 0.05$). Meanwhile, diosmin alone with daily dose of 100mg/kg produced insignificant ($p > 0.05$) change regarding the hepatic MDA in relation to the normal control.

IV. The effect of oral diosmin at daily dose of (100 and 200 mg/kg) for thirty consecutive days, on hepatic Nrf2 and HO-1 levels was

investigated in male albino rats with liver injury induced by oral atorvastatin (40 mg/kg daily) for the same duration. Data are expressed as mean ± SEM (Figures 3,4):

Treatment with atorvastatin led to a marked decline in hepatic Nrf2 and HO-1 levels compared with the control group ($p < 0.05$). Conversely, concurrent administration of diosmin at one hundred and two hundred milligrams per kilogram significantly enhanced the expression of both markers relative to the atorvastatin-only group ($p < 0.05$). Diosmin alone with daily dose of 100mg/kg produced insignificant ($p > 0.05$) change regarding both Nrf2 and HO-1 in relation to the normal control.

V. The impact of oral diosmin, at daily dose of (100 and 200 mg/kg) for thirty days, on histopathological alterations in male albino rats with atorvastatin-induced hepatic injury, administrated at oral daily dose (40 mg/kg) for thirty days, was evaluated using H&E-stained liver sections (Figure 5: A-E):

Hematoxylin and eosin–stained liver sections from the control group revealed preserved hepatic architecture, characterized by eosinophilic hepatocyte cords aligned along well-defined sinusoids, with no signs of necrosis or inflammatory cell infiltration (Figure 5A). In contrast, sections from the atorvastatin-treated group revealed severe central vein congestion, hepatocellular ballooning, cytoplasmic vacuolation, and numerous apoptotic cells with pyknotic nuclei (Figure 5B). In Ator-Dio100-treated group, H&E slides of the liver tissues of the rats showed moderate improvement as the central vein became moderately congested with moderate ballooning and cytoplasmic vacuolation in the hepatocytes. Regression of inflammatory infiltration was noticed (Figure 5C).

On the other hand, in Ator-Dio200-treated group, H&E slides of the liver tissues of the rats showed marked improvement as the hepatocytes showed normal central nuclei and eosinophilic cytoplasm. Minimal lymphocytic infiltration could be seen. The central veins and sinusoids showed mild to no congestion (Figure 5D). In the Dio100-treated group, H&E slides of the liver tissues of the rats exhibited normal hepatic tissue (Figure 5 E).

Table (1): The effect of diosmin (100 and 200 mg/kg/day orally for 30 days) on the serum levels of ALT and the hepatic levels of TNF-α in experimentally-induced liver injury by atorvastatin (40 mg/kg/day orally for 30 days) in male albino rats (mean ± SEM).

Groups	ALT(U/L)	TNF-α (pg./mg)
Control	**46.188 ±1.284**	**116.23 ± 2.120**
Atorvastatin	**253.436 ±2.534*** **	**378.95 ± 4.271*** **
Atorvastatin + diosmin 100	**82.978 ± 3.896 $$$**	**205.54 ± 2.161$$$**
Atorvastatin + diosmin 200	**56.034 ± 1.914 $$$**	**102.51 ± 1.873$$$**
Diosmin 100	**47.372 ± 2.264 #**	**94.32 ± 2.060 *** **

Results are presented as Mean ± SEM (n=7 rats):

Differences were considered statistically significant when $p < 0.05$.

(*) Significant difference versus normal control as (***) P value < 0.001.

($) Significant difference versus atorvastatin group as ($$$) P value < 0.001.

(#) non-significant difference versus the normal control group as p value > 0.05.

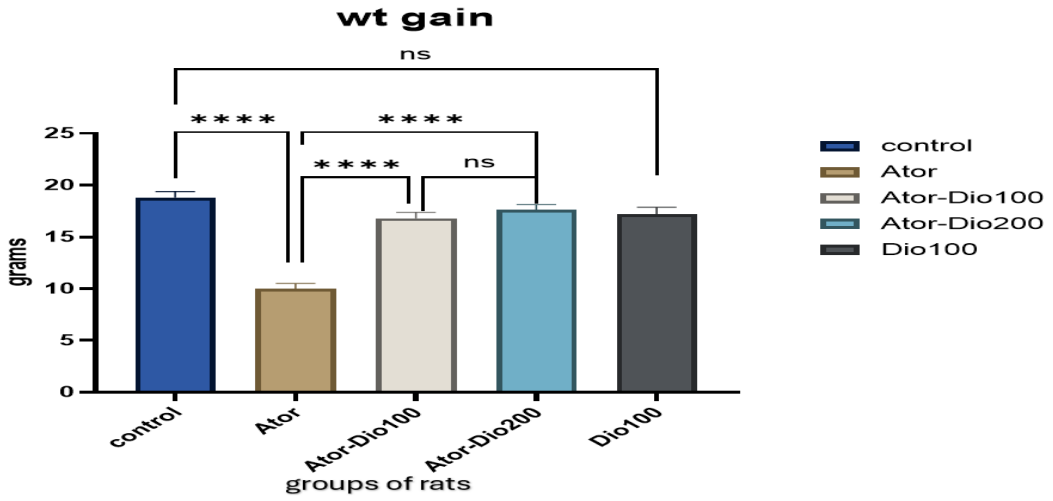

Figure (1): Bar chart showing the effect of diosmin (100 and 200 mg/kg/day orally for 30 days) on the weight gain (Wt. gain) of the rats in experimentally induced liver injury by atorvastatin (40 mg/kg/day orally for 30 days) in the different groups of rats.
Data represented as (Mean ± SEM).
***: p value < 0.001, ns: no statistically significant difference (P value > 0.05).

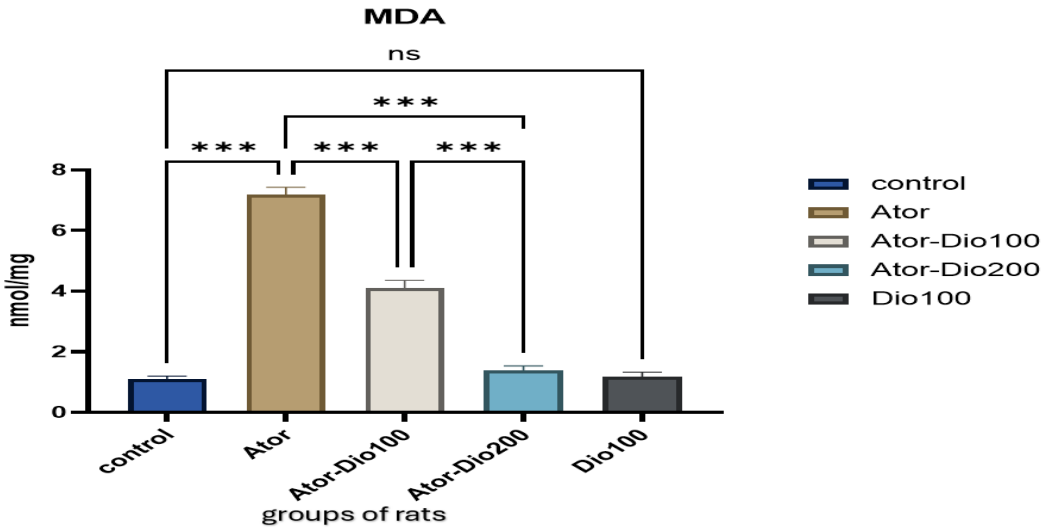

Figure (2): Bar chart showing the effect of diosmin (100 and 200 mg/kg/day orally for 30 days) on the hepatic levels of Malondialdehyde (MDA) in experimentally induced liver injury by atorvastatin (40 mg/kg/day orally for 30 days) in the different groups of rats.
Data represented as (Mean ± SEM).
***: p value < 0.001, ns: no statistically significant difference (P value > 0.05).

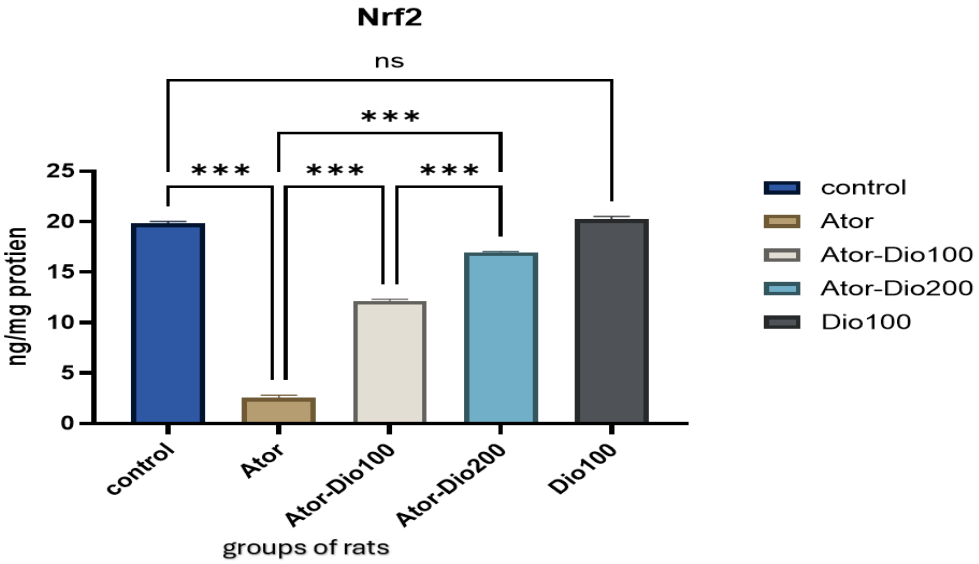

Figure (3): Bar chart showing the effect of diosmin (100 and 200 mg/kg/day orally for 30 days) on the hepatic levels of Nuclear Factor Erythroid-2 (Nrf2) in experimentally induced liver injury by atorvastatin (40 mg/kg/day orally for 30 days) in the different groups of rats.
Data represented as (Mean ± SEM).
***: P value < 0.001, ns: no statistically significant difference (P value > 0.05).

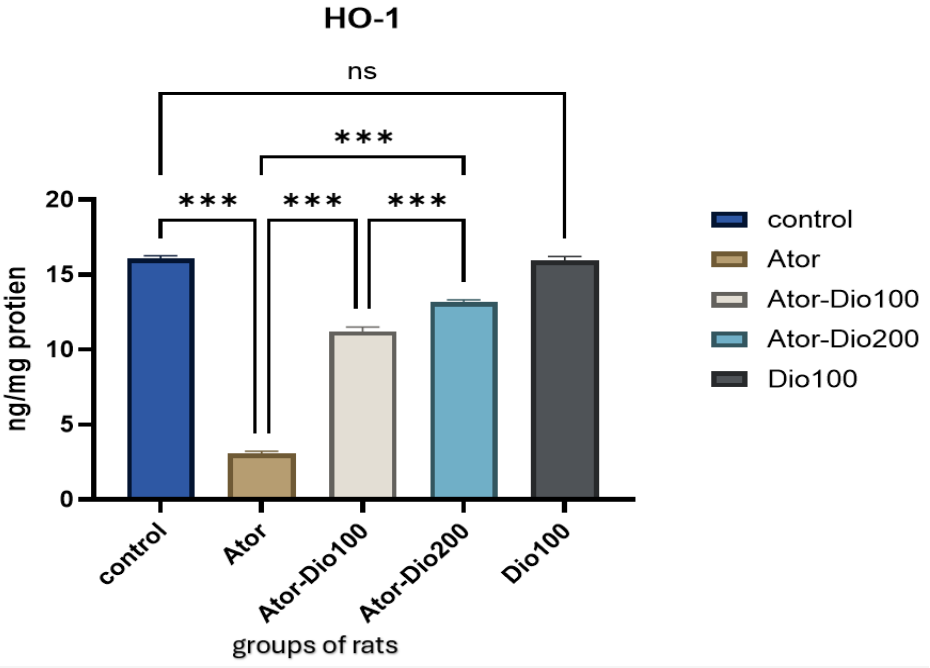

Figure (4): Bar chart showing the effect of diosmin (100 and 200 mg/kg/day orally for 30 days) on the hepatic levels of Heme Oxygenase-1(HO-1) in experimentally induced liver injury by atorvastatin (40 mg/kg/day orally for 30 days) in the different groups of rats.
Data represented as (Mean ± SEM).
***: P value < 0.001, ns: no statistically significant difference (P value > 0.05).

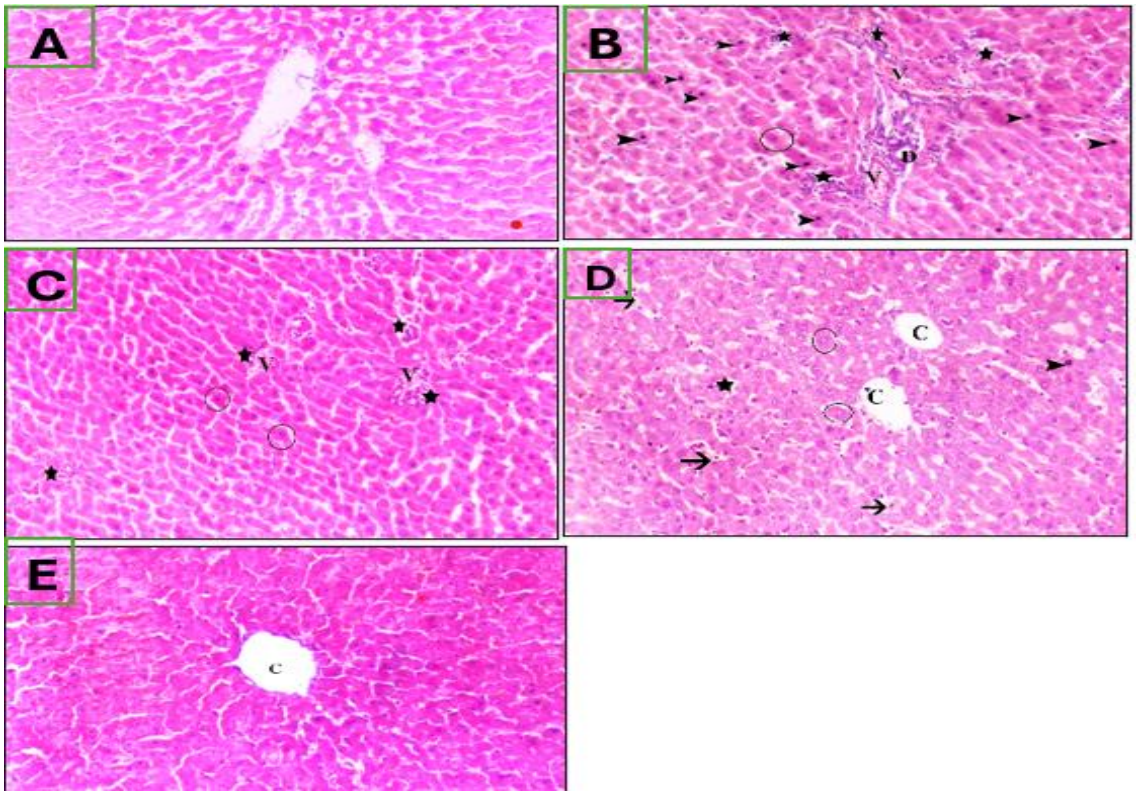

Figure (5): Photomicrographs of liver tissues of the studied groups. **(A) the control group** showing normal liver tissue with cords of eosinophilic hepatocytes separated by liver sinusoids **(B) the Ator-treated group** showing portal tract with dilated congested vascular channel (V) and bile duct proliferation (D) indicating hepatic injury. The portal tract is infiltrated by marked lymphocytic infiltration (star). hepatocytes showed pyknotic dark nuclei indicating hepatocyte apoptosis (arrowhead) (marked Ator damage) **(C) the Ator-Dio100-treated group** showing multiple vascular spaces (V) mostly central veins with vascular congestion surrounded by dilated sinusoids without sinusoidal congestion. Focal lymphocytic infiltration could be seen (star) among hepatocytes with mild ballooning and pyknotic nuclei (mild improvement) **(D) the Ator-Dio200-treated group** showing two central veins without congestion (C) surrounded by dilated sinusoids with mild congestion (arrows). Hepatocytes showed normal central nuclei and eosinophilic cytoplasm (circles) and few hepatocytes showed pyknotic dark nucleus (arrowhead) indicating hepatocyte apoptosis. Minimal lymphocytic infiltration could be seen. (marked improvement). **(E) the Dio100-treated group** showing normal liver tissue with central veins (C) surrounded by cords of eosinophilic hepatocytes separated by liver sinusoids. (H&Ex200).

DISCUSSION

The DILI refers to the unanticipated damage to the liver cells due to the use of drugs or herbs [24]. Atorvastatin is a commonly prescribed agent for the prevention and treatment of cardiovascular disorders; however, its therapeutic value may be compromised by adverse reactions, particularly hepatotoxicity [25]. In contrast, diosmin is associated with minimal side effects and has shown therapeutic promise in conditions such as diabetes, cardiovascular dysfunction, and renal injury [26]. The present work was therefore designed to explore the antioxidant and anti-inflammatory potential of diosmin in mitigating atorvastatin-induced liver toxicity in male albino rats.

We selected rats because they have drug metabolism and hepatic physiological and anatomical features like humans [3]**.** While the male rats were preferred to avoid female estrous cycles and the changes of the hormone levels which cause variable responses to drug-induced injury [27].

Rats in the atorvastatin-treated group exhibited a decrease in weight gain compared with controls. This finding aligns with previous report documenting weight loss in model of atorvastatin-induced liver injury [28]. Administration of diosmin with atorvastatin significantly restored the weight gain level in a dose-dependent manner. Weight loss is an important feature of liver diseases which can be explained by liver damage that causes disruptions in the metabolic processes, loss of appetite and malabsorption [29]. This observation may therefore account for the reduction in body weight observed in this hepatotoxicity model.

Administration of atorvastatin in the current study led to an elevation as regards serum ALT levels in rats. These results are aligned with the results of some authors who reported that atorvastatin (40mg/kg/day) for 30 days had significantly increased the serum level of ALT [30]. Hepatic injury can change the membrane permeability and cellular transport system thereby, leakage of the liver enzyme into the serum [31]**.** accordingly, in this study, atorvastatin damaged the hepatic cell membranes resulting in the leakage of ALT into the serum.

Oral administration of diosmin at one hundred or two hundred milligrams per kilogram daily for thirty days resulted in a dose-dependent decrease in serum ALT levels. These results align with previous reports in which diosmin, given at fifty or one hundred milligrams per kilogram for fourteen days, substantially ameliorated ALT elevations in carbon tetrachloride–induced hepatotoxicity, also demonstrating a dose-dependent effect [16].

The histopathological evaluation in current study of the H&E-stained slides of liver sections showed that atorvastatin caused severe hepatic cells damage. These results corroborate with some authors who reported that alterations in the hepatic architecture, including necrotic hepatocytes, lymphocyte infiltration, and fibrosis in atorvastatin-induced hepatotoxicity [18].

Diosmin also enhanced the histopathological appearance of liver sections in a dose-dependent fashion, as observed in H&E-stained slides. These results coincide with previous research showing that diosmin restored normal hepatic architecture in Wistar rats with isoniazid- and rifampin-induced liver injury [32].

The MDA, a lipid peroxidation product, is widely used as an oxidative stress marker, while TNF-α serves as an indicator of inflammation [33]. In the present study, atorvastatin significantly elevated hepatic MDA and TNF-α levels in the Ator-treated group compared with controls. These results are consistent with [34], who reported increased hepatic MDA and TNF-α in diabetic rats administered atorvastatin (20 mg/kg/day) for 10 days.

In this study, co-administration of diosmin (100 and 200 mg/kg/day for 30 days) with atorvastatin (40 mg/kg/day for 30 days) produced a dose-dependent reduction in hepatic MDA and TNF-α levels. These results are in partial agreement with previous findings where diosmin pretreatment (100 and 200 mg/kg) in doxorubicin-induced nephrotoxicity lowered renal MDA and TNF-α concentrations [35]. Under oxidative stress conditions, inhibitors of NF-κB undergo proteasomal degradation, allowing NF-κB to be released and translocate into the nucleus, where it drives the transcriptional activation of TNF-α [36].

Administration of atorvastatin at a daily oral dose of forty milligrams per kilogram for thirty consecutive days in the current study caused a pronounced decline in hepatic Nrf2 and HO-1 expression when compared with untreated controls. This observation is in line with previous findings reporting suppression of Nrf2 signaling in HepG2 hepatocellular carcinoma cells following atorvastatin exposure [37].

On the other hand, diosmin supplementation at one hundred or two hundred milligrams per kilogram per day successfully restored hepatic Nrf2 and HO-1 levels in a dose-dependent pattern, whether given alone or in combination with atorvastatin. Remarkably, diosmin by itself was capable of enhancing Nrf2 activation, thereby strengthening the cellular antioxidant defense even under normal physiological conditions. These outcomes correspond with earlier evidence showing that diosmin elevated renal Nrf2 and HO-1 expression in rats challenged with gentamicin-induced nephrotoxicity [38].

The cellular defense against oxidative injury relies heavily on antioxidant enzymes, which are largely regulated by the transcription factor Nrf2 [39]. Under normal conditions, Nrf2 is sequestered and degraded through Keap1 control. When this inhibition is disrupted, Nrf2 becomes stabilized, migrates into the nucleus, and activates the transcription of genes responsible for antioxidant protection. This signaling cascade is considered the hallmark of the Nrf2-driven cellular adaptation to oxidative stress [40]. Through its ability to limit intracellular ROS accumulation, Nrf2 attenuates the activation of NF-κB under oxidative stress. This effect preserves IKKβ from proteasomal breakdown, restricts NF-κB entry into the nucleus, and ultimately downregulates TNF-α production [41]. HO-1, another crucial antioxidant enzyme, catalyzes the rate-limiting step of heme metabolism, generating metabolites with potent antioxidant properties [42].

These findings indicate that disruption of oxidant/antioxidant balance is a possible mechanism of atorvastatin-induced liver injury. Diosmin restored the oxidant/antioxidant balance. These results are consistent with some authors who reported that diosmin restored the renal levels of Nrf2 as well as HO-1 in gentamicin-induced nephrotoxicity in rats, consequently, diosmin possess antioxidant effects in this model too [12].

This study provides comprehensive evaluation of diosmin's protective effects against atorvastatin-induced hepatotoxicity by integrating biochemical, molecular, and histopathological assessments. The use of multiple biomarkers (ALT, MDA, TNF-α, Nrf2, and HO-1) alongside histological confirmation strengthens the reliability of the findings. In addition, the dose-dependent design allows better understanding of diosmin's therapeutic potential.

Despite its valuable outcomes, the study was limited by its small sample size, which may affect the generalizability of results. Moreover, the work was restricted to male rats, leaving possible sex-related differences unexplored. The absence of long-term evaluation also limits conclusions about the sustained efficacy and safety of diosmin therapy.

CONCLUSION

This study highlighted that diosmin (100 and 200 mg/kg/day for 30 days) have antioxidant and anti-inflammatory effects, therefore possessing hepatoprotective effects against atorvastatin-induced hepatotoxicity. This diosmin effect was confirmed by improvement of the weight gain along with the serum level of ALT enzyme along with histopathological results. Furthermore, diosmin administration was associated with improvement in the hepatic levels of Nrf2 and HO-1, meanwhile, diosmin decreased the hepatic levels of MDA and TNF-α.

Conflict of Interest & Funding Statement: All authors state that they have non competing interests and confirm that the study did not collect any source of financial support.

Data Availability: Data is accessible from the corresponding author on justified request.

Author Contribution: E.M.M.A. conceptualized the research idea, managed the overall coordination of the project, and served as the corresponding author. She played a central role in drafting and finalizing the manuscript. M.E.K. contributed expert guidance throughout the study and was responsible for overseeing the pharmacological analysis. D.M.A. assisted in refining the methodology and manuscript revision. E.Y.S.E. was involved in gathering

relevant literature and supporting data organization and contributed to data interpretation and manuscript revision. All authors discussed the results and implications and contributed to the final version of the manuscript.

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