



Research Article

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Experimental Evidence Supporting Dauricine as an Anti-Inflammatory Agent in a Mouse Model of NAFLD

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Abstract

Background: Nonalcoholic fatty liver disease (NAFLD) is a multifactorial metabolic disorder characterized by hepatic lipid accumulation, oxidative stress, and chronic inflammation. **Objective:** To evaluate the hepatoprotective effect of dauricine, compared with silymarin, in high-cholesterol diet and D-galactose-induced NAFLD in mice via anti-inflammatory effect. **Methods:** Fifty male Swiss albino mice were divided into five groups: control, NAFLD-induced, silymarin-treated, and dauricine-treated (5 and 10 mg/kg). Serum liver enzymes (ALT, AST) and hepatic IL-6 levels were measured using ELISA, while histopathological changes were assessed using the NAFLD Activity Score (NAS). **Results:** The NAFLD group showed significant elevations in ALT, AST, and IL-6 levels, along with marked hepatic steatosis and inflammation. Treatment with silymarin and dauricine significantly reduced these parameters, with the 10 mg dauricine group showing near-normal enzyme and cytokine levels. Histological analysis confirmed improved hepatic architecture and reduced inflammatory infiltration. **Conclusions:** Dauricine shows potent hepatoprotective effects through modulation of inflammatory pathways, improved liver function, and preservation of tissue integrity. These findings highlight the therapeutic promise of dauricine as a potential adjunct in managing NAFLD.

Keywords: Dauricine; Histopathology; IL-6; Liver enzymes; NAS; NAFLD; Silymarin.

أدلة تجريبية تدعم دوريسين كعامل مضاد للالتهاب في نموذج فأر لمرض NAFLD

الخلاصة

الخلفية: مرض الكبد الدهني غير الكحولي (NAFLD) هو اضطراب أبيض متعدد العوامل يتميز بتراكم الدهون الكبدية، والإجهاد التأكسدي، والالتهاب المزمن. **الهدف:** تقييم تأثير الدوريسين الوقائي للكبد، مقارنة بالسيليمارين، في النظام الغذائي عالي الكوليسترول ومرض الكبد الدهني الناتج عن جالاكتوز في الفئران من خلال التأثير المضاد للالتهابات. **الطرائق:** تم تقسيم خمسين فأر سويسري مصاب بالدهون إلى خمس مجموعات: ضابطة، ناتجة عن مرض NAFLD، معالجة بالسيليمارين، ومعالجة بالدوريسين (5 و 10 ملغ/كغ). تم قياس مستويات إنزيمات الكبد في المصل (ALT، AST) ومستويات IL 6 في الكبد باستخدام ELISA، بينما تم تقييم التغيرات النسيجية المرضية باستخدام درجة نشاط (NAS) NAFLD. **النتائج:** أظهرت مجموعة NAFLD ارتفاعات كبيرة في مستويات ALT و AST و IL-6، إلى جانب تدهور كبدي ملحوظ والتهاب. أدى العلاج بالسيليمارين والدوريسين إلى تقليل هذه المعايير بشكل ملحوظ، حيث أظهرت مجموعة 10 ملغ من الدوريسين مستويات قريبة من الإنزيمات والسيوتوكين. أكد التحليل النسيجي تحسن بنية الكبد وتقليل التسلسل الالتهابي. **الاستنتاجات:** يظهر دوريسين تأثيرات قوية لحماية الكبد من خلال تعديل المسارات الالتهابية، وتحسين وظائف الكبد، والحفاظ على سلامة الأنسجة. تسلط هذه النتائج الضوء على الدور العلاجي للدوريسين كمساعد محتمل في علاج مرض الكبد الوري غير الريبيدي.

***Corresponding author:** Zeena A Hussein. Department of Pharmacology, College of Medicine, Al-Nahrain University, Baghdad, Iraq; Email: zeena.kanada@nahrainuniv.edu.iq**Article citation:** Burhan HQ, Abd AH, Kareem NM, Hussein ZA. Experimental Evidence Supporting Dauricine as an Anti-Inflammatory Agent in a Mouse Model of NAFLD. *Al-Rafidain J Med Sci.* 2026;11(2):10-17. doi: <https://doi.org/10.54133/ajms.v11i2.3101>© 2026 The Author(s). Published by Al-Rafidain University. This is an open access journal issued under the CC BY-NC-SA 4.0 license (<https://creativecommons.org/licenses/by-nc-sa/4.0/>).

INTRODUCTION

Metabolic dysfunction-associated fatty liver disease is a recently introduced nomenclature that describes hepatic steatosis in the presence of metabolic dysfunction. This terminology supersedes the former designation of non-alcoholic fatty liver disease (NAFLD), aiming to capture the underlying metabolic drivers more accurately, including obesity, type 2 diabetes mellitus, and systemic metabolic dysregulation [1]. Epidemiological data indicate that the prevalence of NAFLD (25.7%) and metabolic-associated steatotic liver disease (26.7%) is comparable [2]. Globally, NAFLD affects approximately 25% of the general population, with regional variations: 31.8% in the Middle East, 30.4% in South America, and 13.5% in Africa [3]. NAFLD is defined by the presence of hepatic steatosis in more than 5% of hepatocytes, after

exclusion of other chronic liver diseases and significant alcohol consumption. It encompasses two major subtypes: non-alcoholic fatty liver, characterized by simple steatosis, and non-alcoholic steatohepatitis (NASH), a progressive form associated with hepatocellular injury, inflammation, and an increased risk of fibrosis, cirrhosis, and hepatocellular carcinoma [4]. While liver biopsy remains the diagnostic gold standard, non-invasive imaging modalities—including magnetic resonance imaging (MRI) techniques such as proton density fat fraction (MRI-PDFF) and magnetic resonance spectroscopy, computed tomography (CT), ultrasound (US) with controlled attenuation parameter (CAP), and elastography—offer valuable tools for assessing hepatic fat content and staging fibrosis [2]. Insulin resistance plays a pivotal role in the pathophysiology of NAFLD, driving hepatic lipid

accumulation through enhanced de novo lipogenesis and impaired fatty acid oxidation. These metabolic alterations are accompanied by oxidative stress, inflammatory responses, and activation of lipogenic transcription factors such as sterol regulatory element-binding protein-1c (SREBP-1c), all of which contribute to disease progression [5]. Chronic inflammation and oxidative stress are central mechanisms driving the initiation and progression of NAFLD. Excessive production of reactive oxygen species (ROS) leads to mitochondrial dysfunction and hepatocellular injury, thereby impairing lipid metabolism and promoting hepatic steatosis. Experimental models employing D-galactose to induce aging-related oxidative stress reproduce these pathological features, leading to elevated serum aminotransferases (ALT and AST) and heightened inflammatory responses, thereby resembling NAFLD-like pathology [6, 7]. Among inflammatory mediators, interleukin-6 (IL-6) has emerged as a pivotal cytokine in NAFLD pathogenesis. Secreted primarily by adipose tissue and hepatic Kupffer cells, IL-6 promotes hepatic lipid accumulation, exacerbates insulin resistance, and facilitates the transition from simple steatosis to more advanced liver injury. Elevated IL-6 levels correlate with increased ALT and AST, reflecting hepatocellular damage. IL-6 causes oxidative stress, damages liver cells, and activates inflammation pathways in the liver. Obesity, a major risk factor for NAFLD, further amplifies this inflammatory milieu by promoting adipocyte hypertrophy and dysfunction. Hepatic lipid accumulation, coupled with oxidative stress, compromises the integrity of hepatocyte membranes, leading to the leakage of intracellular enzymes into the circulation and subsequent increases in serum ALT and AST levels. Among these markers, ALT is generally considered more specific for liver injury, whereas elevated AST levels often reflect more extensive hepatic damage and inflammation [9]. This process leads to the release of pro-inflammatory adipokines such as tumor necrosis factor- α (TNF α), IL-1 β , and IL-6, which collectively drive systemic and hepatic inflammation [10]. Histopathological studies demonstrate that increased IL-6 expression is associated with inflammatory cell infiltration, hepatocyte ballooning, lobular inflammation, and fibrosis, underscoring its role in disease progression from simple steatosis to NASH [11]. Dauricine is a natural bisbenzylisoquinoline alkaloid widely distributed among plant species of the family Menispermaceae. Traditionally, it has been utilized for its anti-inflammatory, antiarrhythmic, and neuroprotective properties, particularly in mitigating cerebral damage [12]. Recent studies have highlighted its potent anti-inflammatory activity in vascular endothelial cells, primarily through inhibition of the nuclear factor kappa B (NF- κ B) signaling pathway. This blockade reduces the expression of adhesion molecules such as intercellular Adhesion Molecule-1 (ICAM-1) and Vascular Cell Adhesion Molecule-1 (VCAM-1), as well

as pro-inflammatory cytokines [13]. Mechanistically, dauricine suppresses NF- κ B activation by attenuating reactive oxygen species (ROS) generation, a critical driver of inflammatory signaling. Reduced ROS levels may prevent the inactivation of regulatory proteins such as protein phosphatase 2A (PP2A), thereby maintaining NF- κ B pathway inhibition. Given that oxidative stress and NF- κ B signaling are recognized as two major contributors to the pathogenesis of NAFLD, these findings suggest that dauricine may exert hepatoprotective effects by targeting both pathways [14]. Collectively, these observations position dauricine as a promising candidate for further investigation in the context of NAFLD and related metabolic liver disorders, offering potential therapeutic benefit through its dual modulation of oxidative stress and inflammatory signaling. The study aims to evaluate the hepatoprotective potential of dauricine in an experimental model of NAFLD induced with a high-cholesterol diet and D-galactose, focusing on its anti-inflammatory role.

METHODS

Chemicals and reagents

The chemicals and drugs used in this study came from well-known commercial sources. Cholesterol powder was purchased from Avonchem, UK; D-galactose from Lobachemie, India; silymarin from MEDA, Germany; and dauricine from HyperChem, China. Carboxymethyl cellulose (CMC) from Sigma-Aldrich, USA. The Mouse IL-6 ELISA kit was supplied by ELK Biotechnology (Cat. No. ELK1157).

Experimental animals and study design

Swiss albino male mice (10–12 weeks, 25–30 g) were obtained from the College of Pharmacy, Al-Nahrain University, Baghdad. They were housed in three large cages (15–20 per cage) and acclimatized for one week at the Animal House Unit of the College of Medicine, Al-Nahrain University, Baghdad. Conditions were maintained at 25 ± 2 °C, 45–60% humidity, and a 14/10 h light/dark cycle. Bedding consisted of autoclaved hardwood shavings, with chow and water provided *ad libitum*. Cages were cleaned thrice weekly, bottles daily, and animals monitored for health suitability. The study was conducted using 50 mice, which were randomly allocated into five experimental groups (n= 10) using minimization randomization based on body weight and calculated treatment dose. All animals were maintained under identical environmental and dietary conditions throughout the study period. The groups were defined as follows: The control group received a standard chow pellet diet without additional intervention; The induced group received a high-cholesterol diet (HCD, 2%) combined with D-galactose (100 mg/kg/day, intraperitoneally) for six weeks to induce NAFLD [15, 16]; the Syl group, NAFLD-induced animals treated with

silymarin (40 mg/kg/day, orally) beginning in week 5 of induction [17]; the Dau 5 mg group, NAFLD-induced animals treated with dauricine (5 mg/kg/day, orally) beginning in week 5 of induction [16]; and the Dau 10 mg group, NAFLD-induced animals treated with dauricine (10 mg/kg/day, orally) beginning in week 5 of induction [16]. The induction protocol involved mixing cholesterol powder (2 g per 100 g chow pellet) to prepare the high-cholesterol diet, combined with daily

intraperitoneal administration of D-galactose (100 mg/kg) for six weeks. Treatment interventions with silymarin and dauricine commenced at the start of week 5 and continued for four weeks, resulting in a total 8-week protocol comprising induction and treatment phases (Figure 1). All treatments were administered daily, and animals were handled consistently by the same researcher to minimize experimental bias.

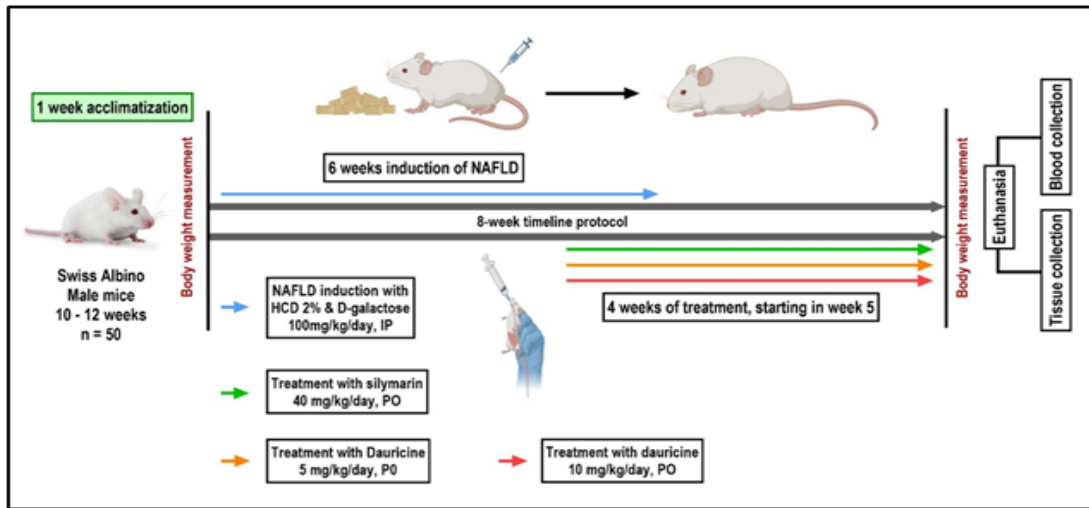


Figure 1: A schematic illustration of the experimental protocol.

Dose calculation

A freshly prepared D-galactose stock (20 mg/mL) was made daily by dissolving 1 g in 50 mL distilled water. Dauricine was suspended in carboxymethyl cellulose (CMC) to deliver 5 or 10 mg/kg doses [16]. The CMC vehicle was prepared by dissolving 0.5 g in 100 mL hot water (~55 °C) with stirring [18]. Silymarin solution (5 mg/mL) was prepared by dissolving 50 mg in 10 mL CMC and administered at 40 mg/kg [17]. All formulations were freshly mixed before administration to ensure uniformity. The correct dose and quantity administered were calculated individually for each animal and adjusted weekly based on changes in animal weight [19]. The following formula was used:

$$\text{Quantity (ml)} = \frac{\text{Animal Wt. (kg)} \times \text{Dose Rate} \left(\frac{\text{mg}}{\text{kg}}\right)}{\text{Preparation Concentration} \left(\frac{\text{mg}}{\text{ml}}\right)}$$

Sample collection and tissue processing

At the end of the 8-week induction and treatment period, animals were weighed and subsequently euthanized using a high dose of ketamine (80 mg/kg, IP) combined with xylazine (10 mg/kg, IP), followed by cervical dislocation to ensure complete sacrifice [20, 21]. Blood samples were collected via cardiac puncture into clot activator tubes for subsequent biochemical analyses. Following blood collection, the liver was excised, rinsed thoroughly with 0.9% normal saline to remove residual

blood and impurities, and dried on sterile filter paper. Portions of liver tissue were stored in Eppendorf tubes for ELISA analysis, and the remaining were fixed in 10% formalin for histopathology.

Liver weight index

Animal weight was recorded just before euthanasia, followed by liver tissue excision and weight measurement to calculate liver index by the following formula [9]:

$$\text{Wt. Index} = \frac{\text{liver Wt. (mg)}}{\text{mice Body Wt. (g)}} \times 100$$

Outcome measurements

Liver tissues stored at -20°C were thawed at ambient temperature before processing. Tissue homogenization and supernatant collection were performed according to Jasim et al., 2025 [19]. Liver homogenates were analyzed for biomarkers relevant to liver tissue injury and inflammation, including IL-6. Blood samples were examined for tissue injury transaminases, including AST and ALT, following the manufacturer's kit instructions. Absorbance was measured using a microplate reader (HumaReader®, USA) at 450 nm, and concentrations were calculated based on standard curves provided with each kit. Following fixation in 10% neutral-buffered

formalin, liver specimens were systematically processed for histological examination and finally stained with hematoxylin and eosin (H&E), then examined under a light microscope (Olympus, Japan) for alterations in tissue architecture across experimental groups as described previously [22, 23]. Histological evaluation of liver sections was performed using the NAFLD Activity Score (NAS), a semiquantitative scoring system that integrates steatosis, lobular inflammation, and hepatocellular ballooning as outlined in [Table 1]. Each feature was scored independently by two blinded pathologists to minimize observer bias. The total NAS (0–8) was calculated as the sum of these parameters. Scores of ≥ 5 were considered diagnostic of NASH, while scores of 0–2 indicated simple steatosis [24].

Table 1: Components of the NAFLD Activity Score (NAS)

Feature	Score range	Criteria
Steatosis	0–3	0: <5% hepatocytes affected 1: 5–33% 2: 34–66% 3: >66%
Lobular Inflammation	0–3	0: No foci 1: 1 focus per 200× field 2: 2–4 foci per 200× field 3: >4 foci per 200× field
Hepatocellular Ballooning	0–2	0: None 1: Few ballooned cells 2: Many cells/prominent ballooning

Ethical considerations

The research idea and manuscript preparation, as well as all procedural work, specifically animal care, were conducted in compliance with the ARRIVE 2.0 guidelines for reporting animal research [25] and the institutional guidelines for animal experiments. The experimental protocol received formal approval from the Institutional Review Board (IRB) of the College of Medicine at Al-Nahrain University (Approval No.: UNCOMIRB202605159, Issue date: 6 November 2025).

Statistical analysis

The sample size included in the study was determined using the statistical power analysis software G*Power 3.1. A priori power analysis was performed, employing the F-test and one-way ANOVA (fixed effects) in accordance with the study design. The sample size was calculated to be 35–40 based on the following input parameters: effect size = 0.6, α -error probability = 0.05, and power ($1-\beta$) = 0.8. The effect size was determined following Cohen's equation and based on outcomes of dauricine in a previous experimental study [25,26]. An additional 10 mice were added to account for errors during the experimental work; thus, the final size was 50. The data are presented in the form of (mean $\pm$ SD).

Multiple group comparisons were done using one-way ANOVA with the Tukey post hoc test for normally distributed variables, and the Kruskal-Wallis test followed by the post hoc Dunn test for the nonparametric (not normally distributed) variables. The level of significance was set at a probability value ($p < 0.05$). Statistical analysis of all data was performed using the Statistical Package for the Social Sciences SPSS 22.0, whereas figures were done using GraphPad Prism, version 11.0.

RESULTS

The study outcomes showed that the control group maintained stable health with no significant changes during the experimental period. By the end of week 2, animals in the induced NAFLD group began to exhibit decreased activity, mild lethargy, and alterations in fur weight and appearance. Occasional aggressive behavior was noted among some animals, though not severe enough to warrant exclusion. Injured animals were promptly treated with 70% ethanol followed by 2% fusidic acid cream (Leo Pharma, Denmark) to prevent opportunistic infections. In contrast, animals in the treated groups (NAFLD + silymarin, NAFLD + dauricine 5 mg, and NAFLD + dauricine 10 mg) demonstrated marked improvement in general health and exhibited fewer behavioral alterations compared to the induced group. No major complications necessitated exclusion from the study. Minor intergroup differences were recorded, and a small number of mortalities occurred across different groups during the study period. The NAFLD-induced group exhibited a significant elevation in serum aminotransferases compared to the control group, indicating marked hepatocellular injury (AST: $p < 0.0001$; ALT: $p = 0.007$). Treatment with silymarin and dauricine (both doses) significantly attenuated these alterations, as evidenced by reduced AST and ALT levels relative to the untreated induced group. Animals in the dauricine 10 mg group demonstrated enzyme levels approaching those of the control group, suggesting a pronounced hepatoprotective effect (Figure 2A and B). The NAFLD-induced group demonstrated a significant elevation in hepatic IL-6 levels compared with the control group ($p < 0.0001$), indicating a pronounced pro-inflammatory state within liver tissue. Treatment with silymarin and dauricine (5 and 10 mg) significantly reduced hepatic IL-6 concentrations relative to the untreated induced group. Importantly, the dauricine 10 mg group exhibited the most pronounced reduction, with IL-6 levels approaching those of the control group, thereby suggesting a strong anti-inflammatory effect (Figure 2C). Animals in the induced-only group showed a significant weight increase ($p < 0.05$) by the end of the induction period (6 weeks of high HCD 2% and D-galactose administration) compared to the starting point of the experiment.

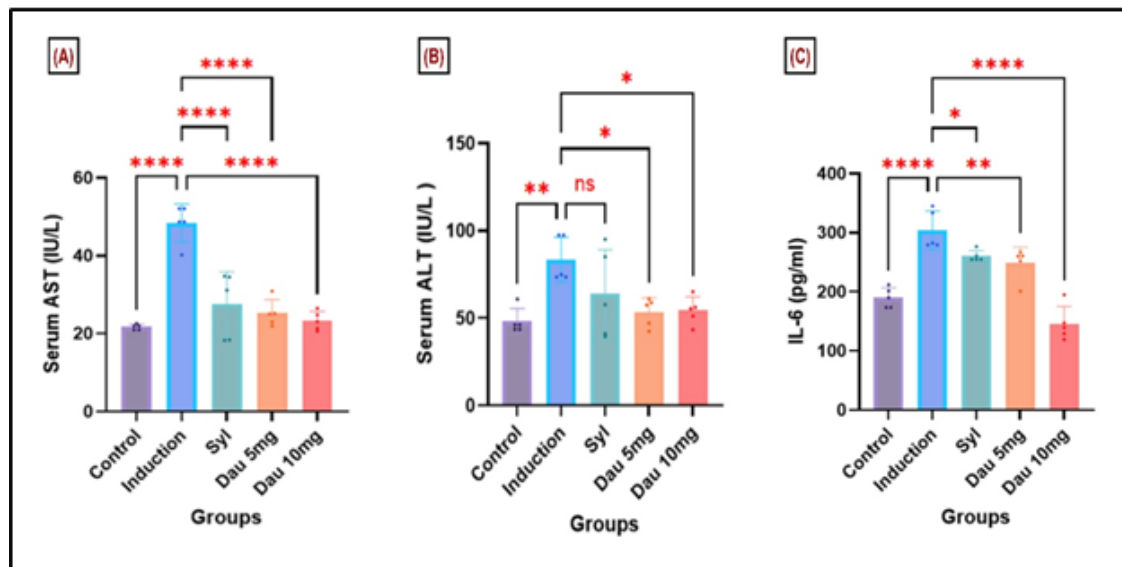


Figure 2: Changes in the levels of serum liver function parameters. (A) AST, (B) ALT, (C) tissue homogenate inflammatory mediator IL-6 among experimental animal groups. Results are expressed as mean $\pm$ SD, n= 10, significant level was set at ($p < 0.05$). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$, ns = non-significant.

In contrast, animals in the treated groups (silymarin and dauricine) did not show a statistically significant increase in weight by the end of the experimental timeline compared to the starting point and the induced-only group, as shown in Figure 3B. This is complemented by the statistical outcomes of the liver weight index, where the induced-only group expressed a significantly higher index ($p < 0.05$) compared to the control group. The weight index was improved in the treated groups with a marked reduction in the animal group that received Dau 10 mg/kg ($p < 0.001$) over those that received Dau 5 mg/kg and Syl 40 mg/kg (standard), as shown in Figure 3C. During histopathological evaluation, three liver samples from each experimental group were excluded due to technical issues, including tissue damage during excision, improper formalin fixation, slide preparation errors, and inadequate staining. These exclusions were carefully considered to avoid compromising statistical validity, ensuring that the remaining samples provided reliable and representative data for analysis. The histological analysis revealed that a liver segment from the control group included normal hepatic tissue, including the portal triad (arrow) and central vein. In contrast, the liver portion of the induced-only group demonstrated portal lymphocytic inflammation (arrow). The treated groups with silymarin and dauricine (5 and 10 mg/kg) showed liver tissue sections with near-normal architecture, no steatosis, no ballooning degeneration, and a minimal localized lymphocytic inflammatory infiltrate near the central vein (arrow) (Figure 3A). Histological examination revealed a significant increase in the NAS in the NAFLD-induced group compared with the control group ($p < 0.0001$) (Figure 3D), reflecting marked hepatic steatosis and tissue injury. Treatment with silymarin and dauricine (5 and 10 mg) significantly improved hepatic architecture, as evidenced by reduced

NAS values relative to the untreated induced group (Figure 3A and 3D). Notably, the dauricine 10 mg group demonstrated the greatest reduction in NAS, with scores approaching those of the control group, indicating a strong hepatoprotective effect.

DISCUSSION

Nonalcoholic fatty liver disease is a prevalent chronic liver disorder characterized by the excessive accumulation of fat within hepatocytes [27]. It represents a multifactorial metabolic condition in which hepatic lipid deposition is accompanied by oxidative stress and persistent inflammation. The transition from simple steatosis to NASH is strongly influenced by pro-inflammatory mediators, particularly IL-6. IL-6 plays a pivotal role in driving hepatic injury and disease progression, with elevated expression levels being closely associated with hepatocellular damage, inflammatory responses, and the development of insulin resistance [28]. The experimental model of NAFLD was induced in mice through a six-week regimen of an HCD combined with D-galactose administration. This regimen reliably induces oxidative stress, inflammation, and steatosis, thereby replicating key pathological features of NAFLD. D-galactose has been shown to activate the p38 MAPK–NRF2–HO-1 (mitogen-activated protein kinase–Nuclear factor erythroid 2-related factor 2–heme oxygenase-1) signaling pathway in hepatic tissue, contributing to oxidative stress responses and inflammation [29, 30]. Mice receiving 2% cholesterol exhibited elevated serum ALT and increased hepatic cholesterol levels, consistent with NAFLD progression driven by free cholesterol accumulation [15].

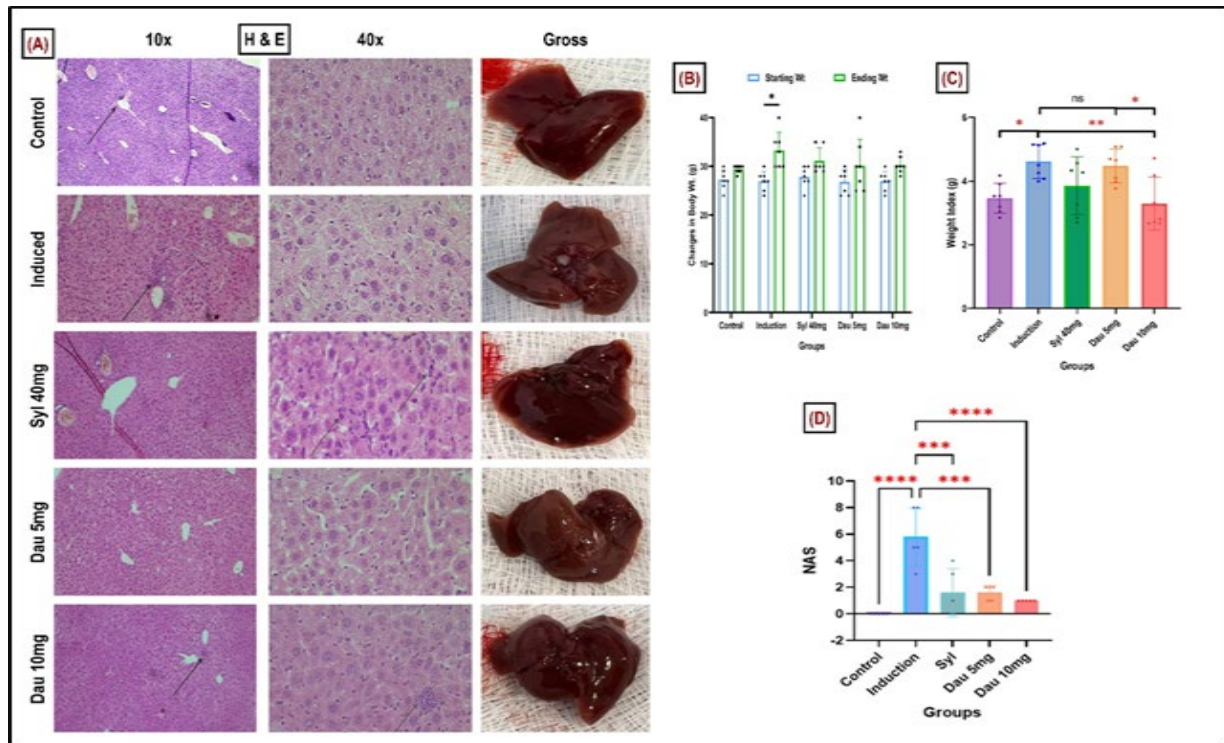


Figure 3: Changes in structural features observed in liver tissue section grossly and microscopically (A), changes in body weight (B), liver weight index (C), the NAFLD activity score (NAS) (D) among experimental animal groups. Results are expressed as mean $\pm$ SD (n=7/group after exclusion); the significance level was set at $p < 0.05$. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$, ns = non-significant.

Hepatocellular injury in the NAFLD model was evidenced by significant elevations in serum ALT and AST in the induced group compared with the healthy (control) group, as supported by previous research [11]. In the present study, the NAFLD group exhibited elevated liver function parameters accompanied by distinct histopathological alterations, including inflammatory cell infiltration and hepatic injury. These findings suggest that fatty liver disease is associated with hepatocellular membrane damage and progressive inflammation. Histological examination further confirmed hepatic tissue injury, consistent with the biochemical data. Hepatic IL-6 levels also increased, confirming an inflammatory response. These findings align with previous reports of diet-induced NAFLD showing aminotransferase elevation, steatosis, and inflammation [31]. The observed increase in IL-6 levels in the diseased group may reflect activation of inflammatory pathways, particularly NF- κ B signaling, which drives the production of pro-inflammatory cytokines during NAFLD progression. Elevated IL-6 has been reported to correlate positively with the severity of hepatic inflammation and fibrosis, underscoring its role as a key mediator in disease advancement [8]. Elevated serum levels of ALT and AST are widely recognized as key biochemical indicators of hepatocellular injury in NAFLD. In the present study, the NAFLD-induced group demonstrated significant elevations in liver function enzymes, specifically ALT and AST, compared with the control group. These increases are indicative of hepatocellular injury and hepatic dysfunction associated

with fatty liver progression. The elevation of liver enzymes may be linked to the production of inflammatory cytokines, particularly IL-6, which contributes to oxidative stress and hepatocyte damage during the course of NAFLD. The histopathological analysis indicated that there was inflammation and damage to the liver tissue, which matches the biochemical results. Chronic inflammation promotes cellular breakdown and increases membrane permeability, thereby facilitating the release of intracellular enzymes into circulation. Together, these results highlight the interplay between biochemical markers and inflammatory pathways in the progression of NAFLD. Dauricine and silymarin treatment markedly attenuated these abnormalities, with dauricine (10 mg/kg) producing the greatest improvement, normalizing ALT, AST, and IL-6 levels toward control values. Silymarin similarly reduced liver enzyme elevations and histopathological changes, consistent with prior studies demonstrating its hepatoprotective and anti-inflammatory effects in diet-induced NAFLD models [32,33]. Treatment with silymarin resulted in a marked improvement in liver enzyme levels (AST and ALT) and attenuation of histopathological abnormalities, demonstrating its hepatoprotective potential. These beneficial effects are likely attributable to its antioxidant and anti-inflammatory properties, including free radical scavenging and suppression of pro-inflammatory mediators such as IL-6. By mitigating oxidative stress and dampening cytokine-driven inflammation, silymarin helps preserve hepatocyte integrity and reduce hepatic

injury. Previous studies have indicated that silymarin protects the liver and has antioxidant effects by lowering changes in blood chemistry and liver health caused by diet-related NAFLD. In another investigation, silymarin alleviated lipid metabolic abnormalities and hepatic steatosis, though ALT and AST changes were not statistically significant, likely reflecting differences in injury severity, dosage, induction duration, or model design [34]. In the present study, silymarin further supported its hepatoprotective role by improving liver enzyme levels and reducing histopathological abnormalities, consistent with its reported benefits in NAFLD models. The improved histological findings, characterized by reduced inflammatory infiltration and tissue damage, further corroborate the protective role of silymarin against the progression of NAFLD [35]. The doses for dauricine selected for the current study were based on a previous report demonstrating beneficial effects without notable toxicity in experimental animal models across a wide dosing range (1–12 mg/kg). However, toxic manifestations have been reported at substantially higher doses, such as 150 mg/kg [16]. Dauricine treatment produced marked improvements in liver morphology, reduced inflammatory infiltration, and normalized liver function markers in this study. These outcomes align with previous experimental evidence supporting the anti-inflammatory and hepatoprotective properties of dauricine. Notably, dauricine has been shown to attenuate CCl₄-induced liver injury in mice [35], further reinforcing its potential as a hepatoprotective agent. These beneficial effects are largely attributed to its anti-inflammatory activity, particularly through inhibition of NF- κ B signaling, which suppresses the production of pro-inflammatory cytokines such as IL-6 and TNF- α . Previous studies have demonstrated that dauricine attenuates inflammatory responses and decreases cytokine production via NF- κ B pathway inhibition [13]. The improvement in histopathological findings observed in the present study was accompanied by reductions in inflammatory markers and liver enzyme levels, suggesting that suppression of inflammation plays a central role in halting NAFLD progression. These findings are consistent with previous evidence regarding the anti-inflammatory effects of dauricine and further support its potential in experimentally induced NAFLD [36]. Taken together, the hepatoprotective effects of silymarin and dauricine appear to be mediated through modulation of inflammatory cytokines—particularly IL-6—resulting in improved hepatic function and preservation of tissue architecture. These findings highlight the therapeutic potential of targeting inflammatory pathways in the management of NAFLD. Therefore, both silymarin and dauricine appear to exert hepatoprotective effects through modulation of inflammatory pathways, attenuation of oxidative stress responses, and suppression of NF- κ B signaling. These mechanisms collectively reduce the production of pro-inflammatory cytokines

such as IL-6, thereby alleviating hepatocellular injury and limiting disease progression in NAFLD. The consistency of these findings with previous reports on the anti-inflammatory and antioxidant properties of silymarin and dauricine in experimental liver injury models further supports their therapeutic potential [13,37].

Study Limitations

This study was conducted using an experimental animal model, which may not fully replicate the complex metabolic and inflammatory interactions observed in human NAFLD. The relatively short duration and limited sample size could restrict the generalizability of the findings. Additionally, only selected biochemical and histological parameters were assessed, without evaluation of molecular markers or long-term outcomes, which was due to the limited funding and resources. Future research should include extended treatment periods and mechanistic investigations to validate these results in clinical settings.

Conclusion

Dauricine exerts a significant dose-response hepatoprotective effect in NAFLD, as evidenced by improvements in liver enzyme levels, attenuation of histopathological abnormalities, and reductions in inflammatory markers. These effects appear to be mediated by modulation of inflammation, as observed by decreased production of pro-inflammatory cytokine IL-6. The consistency of these findings with previous reports highlights the therapeutic potential of these phytochemicals in mitigating hepatocellular injury and preserving liver function.

Conflict of interests

The authors declared no conflict of interest.

Funding source

The authors did not receive any source of funds.

Data sharing statement

The data that supports the findings of this study are available from the corresponding author upon a reasonable request.

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