



Article

Dexpanthenol Attenuates High-Fructose Corn Syrup-Induced Hepatic Injury in Young Adult Rats by Modulating Oxidative Stress, Apoptosis, and Inflammasome-Associated Pyroptotic Signaling

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Abstract

Excessive intake of high-fructose corn syrup (HFCS) contributes to pediatric metabolic dysfunction-associated steatotic liver disease, but the mechanisms linking fructose exposure to inflammatory cell death remain incompletely defined. This study investigated whether dexpanthenol (DEX) attenuates HFCS-induced liver injury by modulating oxidative stress, apoptosis, and nucleotide-binding domain-like receptor protein 3 (NLRP3) inflammasome-associated pyroptotic signaling. Thirty-two young adult male Wistar rats were assigned to control, HFCS, HFCS+DEX, and DEX groups ($n = 8$ each). HFCS-induced liver injury was established with 20% HFCS-55 in drinking water for 8 weeks. DEX (500 mg/kg/day, intraperitoneally) was administered from the end of week 4 to week 8. Liver tissues were assessed by histopathology; immunohistochemistry for caspase-3, malondialdehyde, and proliferating cell nuclear antigen; biochemical measurement of total antioxidant and oxidant status; and RT-qPCR analysis of *Nlrp3*, *caspase-1*, *gasdermin D*, and *interleukin-1 β* . HFCS exposure caused steatosis, inflammation, and necrosis; increased histopathological scores; enhanced caspase-3, malondialdehyde, and proliferating cell nuclear antigen expression; elevated total oxidant status; and markedly upregulated inflammasome-related genes. Total antioxidant status did not differ among groups. DEX significantly improved hepatic architecture; reduced immunohistochemical markers of oxidative stress, apoptosis, and injury-associated proliferation; and downregulated NLRP3, caspase-1, gasdermin D, and interleukin-1 β expression. These findings suggest that DEX attenuates HFCS-induced liver injury through a multi-target mechanism involving suppression of oxidative damage, apoptosis, and inflammasome-associated pyroptotic signaling in young adult rats.

Keywords: metabolic dysfunction-associated steatotic liver disease; NLRP3 inflammasome; pyroptosis; gasdermin D; lipid peroxidation; young adult rat model



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1. Introduction

Metabolic dysfunction-associated steatotic liver disease (MASLD) has emerged as the most common chronic liver disease in both adults and children, and its burden continues to rise in parallel with pediatric obesity and unhealthy dietary patterns [1]. Among these dietary factors, excessive fructose intake, particularly through sugar-sweetened beverages and high-fructose corn syrup (HFCS)-containing processed foods, has been increasingly linked to hepatic steatosis, dyslipidemia, insulin resistance, and broader cardiometabolic risk in children and adolescents [2,3]. Recent experimental data further suggest that fructose-related liver injury is not merely a consequence of caloric excess but also reflects active metabolic and inflammatory disturbances, including oxidative stress, hepatic lipid accumulation, and inflammasome-associated signaling [2,4]. Because HFCS consumption is disproportionately high among children and adolescents and pediatric MASLD is increasing worldwide, understanding early pathogenic mechanisms in juvenile liver tissue is of particular translational importance.

Beyond metabolic dysregulation, growing evidence indicates that inflammatory signaling pathways play a pivotal role in the progression of fructose-induced liver injury. In particular, activation of the nucleotide-binding domain-like receptor protein 3 (NLRP3) inflammasome has emerged as a central mechanism linking metabolic stress to hepatic inflammation [5,6]. The NLRP3 inflammasome, a multiprotein complex activated by cellular stress signals such as reactive oxygen species and lipid accumulation, promotes the activation of caspase-1 (CASP1), leading to the maturation and release of pro-inflammatory cytokines, including interleukin-1 β (IL-1 β) [6,7]. In addition, CASP1 activation induces cleavage of gasdermin D (GSDMD), triggering pyroptosis, a highly inflammatory form of programmed cell death that amplifies tissue injury and immune activation in the liver [7,8]. Recent experimental and clinical studies have demonstrated that NLRP3-mediated pyroptosis contributes significantly to the development of hepatic steatosis, inflammation, and progression toward steatohepatitis, highlighting this pathway as a promising therapeutic target [5,8].

Dexpanthenol (DEX; D-panthenol) is the biologically active R-enantiomer of panthenol, with the molecular formula $C_9H_{19}NO_4$ and a molecular weight of 205.25 g/mol. Its systematic chemical name is (2R)-2,4-dihydroxy-N-(3-hydroxypropyl)-3,3-dimethylbutanamide. Structurally, DEX is the alcohol analogue of D-pantothenic acid (vitamin B5), in which the terminal carboxylic acid group is replaced by a primary alcohol group. Following biological oxidation to pantothenic acid, it contributes to the biosynthesis of coenzyme A. DEX has demonstrated antioxidant, anti-inflammatory, and cytoprotective effects in various experimental models of tissue injury [9–11]. Previous studies have demonstrated that DEX reduces oxidative stress by enhancing endogenous antioxidant defenses, limiting lipid peroxidation, and attenuating cellular damage in liver, kidney, and intestinal injury models [10]. In addition, DEX has been reported to modulate apoptotic signaling pathways, including caspase activation and the balance between BCL2-associated X protein (BAX) and B-cell lymphoma 2 (BCL2), thereby contributing to cellular survival and tissue regeneration [12]. However, despite these well-established protective properties, its potential role in regulating inflammasome activation and pyroptosis, particularly in the context of fructose-induced hepatic injury, remains largely unexplored [9,12]. In addition to canonical pyroptotic signaling, fructose-induced hepatic injury involves closely interconnected oxidative, apoptotic, and regenerative responses. MDA reflects lipid peroxidation and oxidative membrane damage, which may act as an upstream trigger for inflammasome activation [13,14]. Caspase-3 (Cas-3), a key executioner of apoptosis, provides complementary information on non-pyroptotic programmed cell death occurring in parallel with inflammasome-mediated injury [15]. Proliferating cell nuclear antigen (PCNA), as a marker

of cellular proliferation and reparative activity, may indicate the regenerative response of hepatocytes following metabolic and inflammatory damage [16].

In this study, we hypothesized that DEX ameliorates HFCS-induced liver injury in association with reduced *Nlrp3*, *Casp1*, *Gsdmd*, and *Il-1 β* related transcriptional signaling, together with attenuation of lipid peroxidation, apoptosis, and injury-associated proliferative responses, as reflected by MDA, Cas-3, and PCNA immunoreactivity.

2. Results

2.1. Histopathological Examination

Histopathological examination showed preserved hepatic architecture in the control and DEX groups, with regularly arranged hepatocyte cords, intact sinusoids, and no evident inflammatory infiltration, steatosis, necrosis, or vascular congestion. In contrast, liver sections from the HFCS group exhibited sinusoidal dilatation, hyperemia, focal hemorrhage, inflammatory cell infiltration, hepatocellular ballooning, necrosis, and lipid accumulation, particularly in the centrilobular regions. The HFCS+DEX group showed substantially less inflammatory infiltration, lipid accumulation, and hepatocellular necrosis than the HFCS group. Representative histopathological findings for all experimental groups are shown in Figure 1.

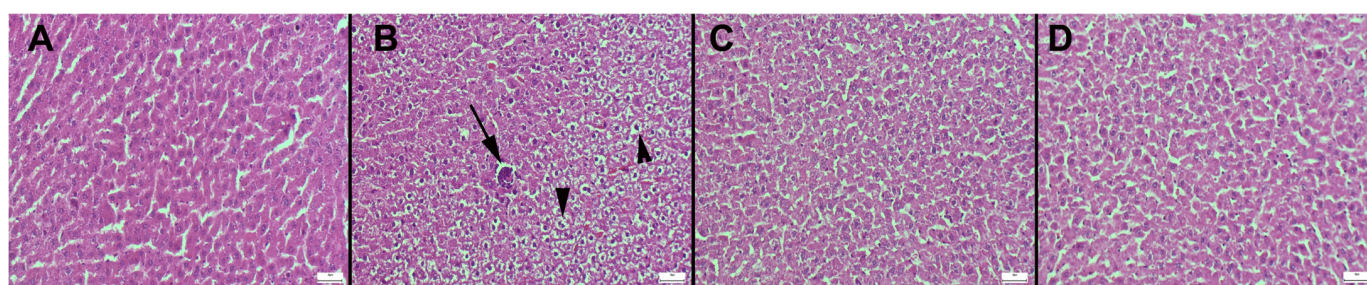


Figure 1. Representative histopathological images of liver tissues from experimental groups. (A) Control group showing normal hepatic architecture with intact hepatocytes and sinusoids. (B) HFCS group displaying inflammatory cell infiltration (arrow) and pronounced lipid accumulation (lipidosis) (arrowheads). (C) HFCS+DEX group demonstrating substantially reduced pathological alterations, including decreased inflammation and lipid deposition. (D) DEX group exhibiting normal liver histology, comparable to controls. Hematoxylin and eosin (HE) staining; scale bars = 50 μ m.

Semi-quantitative analysis demonstrated that hyperemia, hemorrhage, inflammation, necrosis, and lipidosis scores were significantly higher in the HFCS group than in the control group, with the exact adjusted p values presented in Figure 2. Compared with the HFCS group, DEX treatment significantly reduced hyperemia (adjusted $p = 0.012$), hemorrhage (adjusted $p < 0.001$), inflammation (adjusted $p = 0.012$), and lipidosis scores (adjusted $p = 0.011$). Although the mean necrosis score was lower in the HFCS+DEX group than in the HFCS group, this difference did not reach statistical significance (adjusted $p = 0.159$). No significant differences were observed between the control and DEX groups for any histopathological parameter (Figure 2).

These findings demonstrate that HFCS induces severe hepatocellular injury, while DEX effectively ameliorates these alterations, indicating a strong hepatoprotective effect (Figure 2).

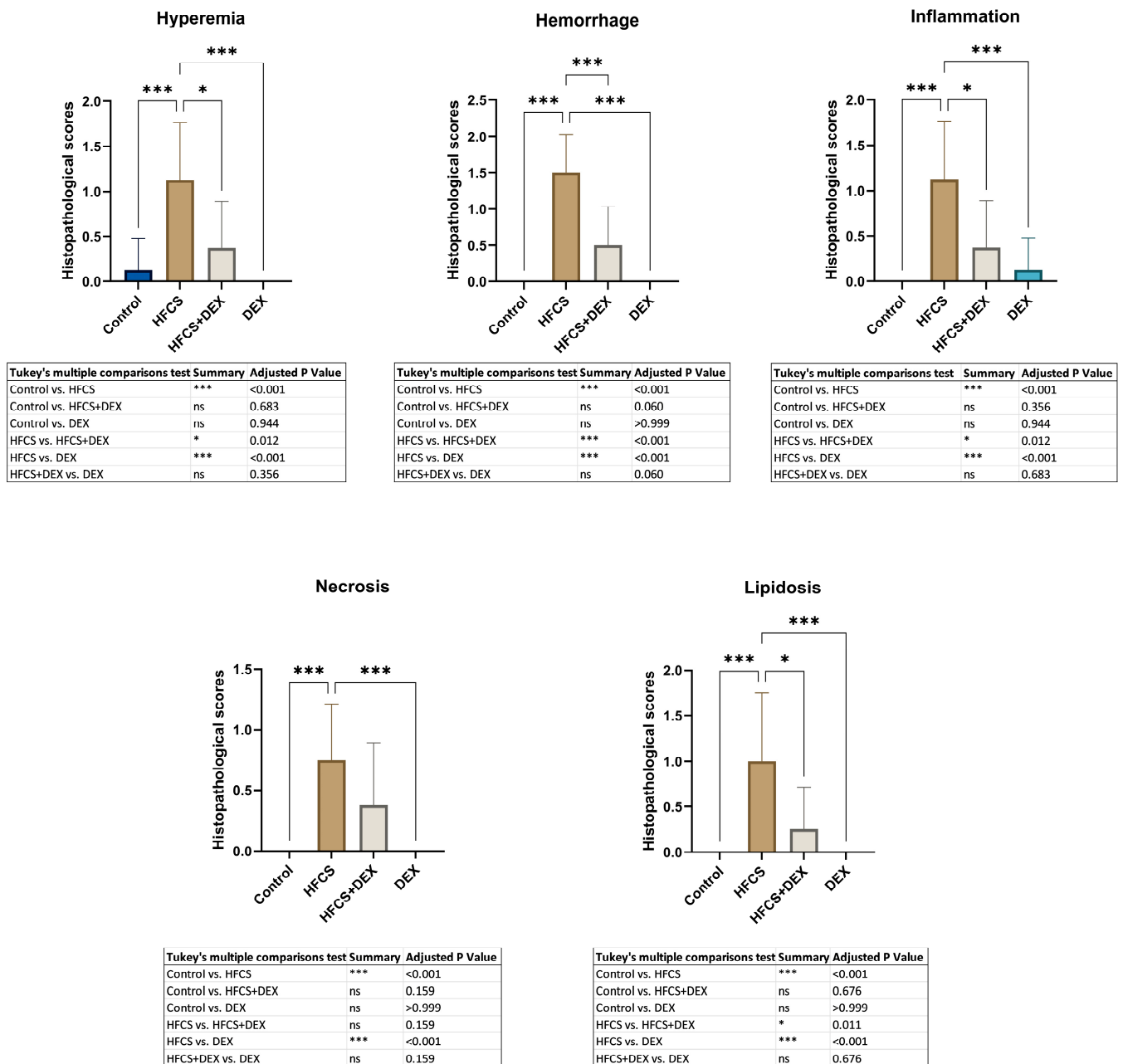


Figure 2. Hyperemia, hemorrhage, inflammation, necrosis, and lipidosis scores were increased in the HFCS group compared with the control group. DEX treatment significantly reduced hyperemia, hemorrhage, inflammation, and lipidosis scores compared with the HFCS group. Although the necrosis score was numerically lower in the HFCS+DEX group, the difference was not statistically significant. The DEX-only group showed scores comparable to those of the control group. Bars represent mean $\pm$ SD. Tables below the graphs present adjusted p values obtained using one-way ANOVA followed by Tukey's multiple-comparisons test. Statistical significance is indicated as $*p < 0.05$ and $***p < 0.001$. HFCS, high-fructose corn syrup; DEX, dextranthenol.

2.2. Immunohistochemical Examination

Immunohistochemical analysis revealed minimal expression of Cas-3, MDA, and PCNA in the control and DEX groups, consistent with basal levels of apoptosis, oxidative stress, and cellular proliferation in normal liver tissue.

In contrast, the HFCS group exhibited significantly increased immunoreactivity for all three markers compared with the control group. Cas-3 expression was markedly elevated, indicating enhanced apoptotic activity ($p < 0.001$). Similarly, MDA expression was significantly increased, reflecting heightened lipid peroxidation and oxidative stress ($p < 0.001$). PCNA expression was also upregulated, suggesting a compensatory proliferative response to hepatocellular injury ($p < 0.01$). Following DEX treatment, the HFCS+DEX group showed significantly reduced expression of Cas-3, MDA, and PCNA compared with the HFCS group. This reduction was significant for Cas-3 ($p < 0.001$), MDA ($p < 0.001$), and PCNA ($p < 0.01$). No significant differences were observed between the control and DEX groups (Figure 3).

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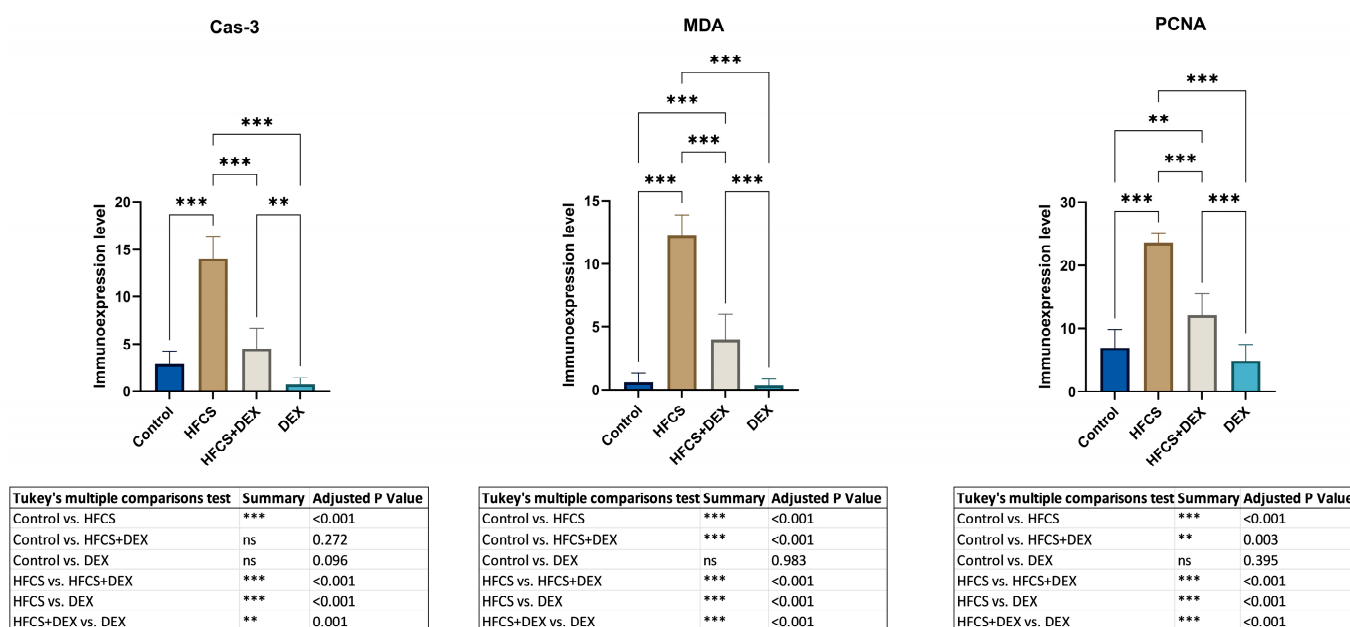


Figure 3. Immunohistochemical evaluation of apoptotic, oxidative stress, and proliferative markers in liver tissues. Immunohistochemical analysis demonstrated significant alterations in Caspase-3 (Cas-3), malondialdehyde (MDA), and proliferating cell nuclear antigen (PCNA) expression across experimental groups. The HFCS group exhibited markedly increased immunoreactivity levels of Cas-3, MDA, and PCNA compared to the control group, indicating enhanced apoptosis, oxidative stress, and hepatocellular proliferation. In contrast, DEX treatment (HFCS+DEX group) significantly reduced the expression of these markers, reflecting attenuation of hepatocellular injury. The DEX-only group showed expression levels comparable to controls. Bar graphs represent mean $\pm$ SD of immunoreactivity levels for each marker. Tables below the graphs present the adjusted p values obtained using one-way ANOVA followed by Tukey's multiple-comparisons test. Statistical significance is indicated as ** $p < 0.01$ and *** $p < 0.001$. Immunoreactivity level represents the percentage of positively stained cells quantified using ImageJ software in ten randomly selected fields per animal at $\times 40$ objective magnification. Field-level measurements were averaged to obtain a single value for each animal, which was used as the experimental unit for statistical analysis. **Abbreviations:** HFCS, high-fructose corn syrup; DEX, dexamethasone; Cas-3, caspase-3; MDA, malondialdehyde; PCNA, proliferating cell nuclear antigen.

Immunoreactivity was predominantly localized in the cytoplasm of hepatocytes, with occasional staining in inflammatory and biliary epithelial cells. Representative immunohistochemical images showed intense hepatocellular staining for Cas-3, MDA, and PCNA in the HFCS group, whereas staining was substantially weaker in the HFCS+DEX group and minimal in the control and DEX groups. These findings indicate that DEX attenuates HFCS-induced apoptosis, oxidative stress, and injury-associated proliferative activity. These results highlight the cytoprotective and anti-apoptotic properties of DEX in experimental liver injury (Figure 4).

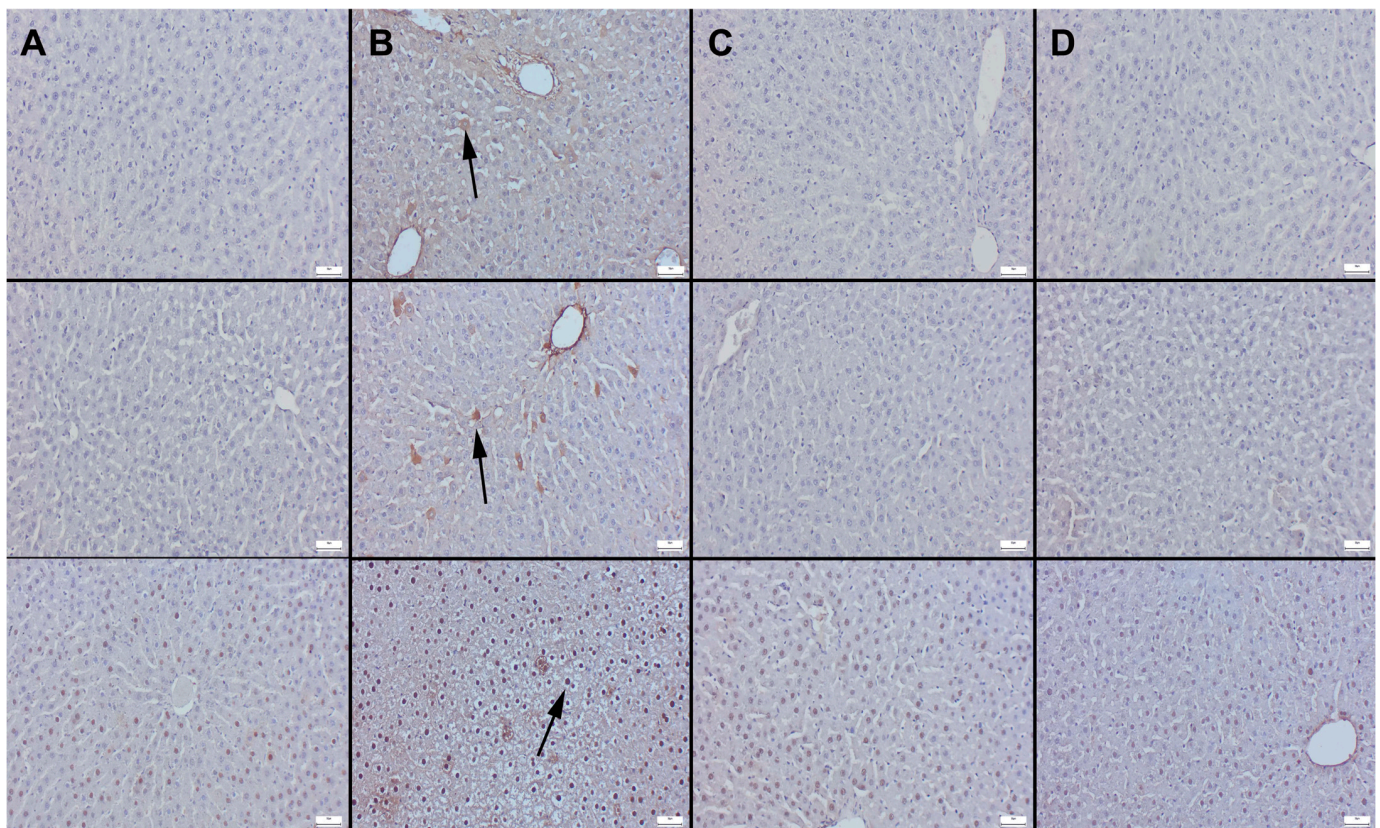


Figure 4. The columns, from left to right, represent the control, HFCS, HFCS+DEX, and DEX groups, respectively. The first, second, and third rows show caspase-3 (Cas-3), malondialdehyde (MDA), and proliferating cell nuclear antigen (PCNA) immunostaining, respectively. (A) Control group showing negative to minimal expression of Cas-3, MDA, and PCNA, indicating basal levels of apoptosis, oxidative stress, and proliferation. (B) HFCS group exhibiting markedly elevated expression in hepatocytes (arrows), reflecting increased apoptotic activity, oxidative damage, and proliferative response. (C) HFCS+DEX group demonstrating significant reduction in marker expression, suggesting a protective effect of DEX against HFCS-induced hepatic injury. (D) DEX group showing slight to negative expression, comparable to controls. Immunostaining performed using the HRP/DAB detection system; scale bars = 50 μ m.

These findings suggest that DEX exerts a hepatoprotective effect against HFCS-induced liver injury, as evidenced by the restoration of normal histological architecture, reduction in inflammatory cell infiltration, and decreased expression of apoptotic, oxidative, and proliferative markers. Collectively, these results indicate that DEX effectively mitigates hepatocellular stress and damage, highlighting its potential therapeutic role in protecting liver tissue from chemically or toxin-induced injury.

2.3. Gene Expression Analysis (RT-qPCR Findings)

Quantitative gene expression analysis demonstrated marked activation of inflammasome- and pyroptosis-related signaling following HFCS exposure. Specifically, *Nlrp3* expression was markedly elevated in the HFCS group, indicating activation of the inflammasome pathway (Figure 5). Similarly, *Casp1* expression was significantly increased, supporting the involvement of *Casp1*-related inflammatory signaling. *Gsdmd* expression was also upregulated, suggesting activation of pyroptosis-associated cell death mechanisms (Figure 5). In parallel, *Il-1β* expression was significantly elevated, indicating an amplified inflammatory response (Figure 5). Importantly, DEX treatment significantly suppressed the expression of all these genes in the HFCS+DEX group ($p < 0.01$ – 0.001), bringing their levels closer to those observed in the control group. No significant differences were observed between the control and DEX-only groups. These results indicate that HFCS-induced liver injury is strongly associated with activation of the NLRP3 inflammasome and pyroptosis pathways, while DEX exerts a modulatory effect by suppressing these molecular mechanisms (Figure 5).

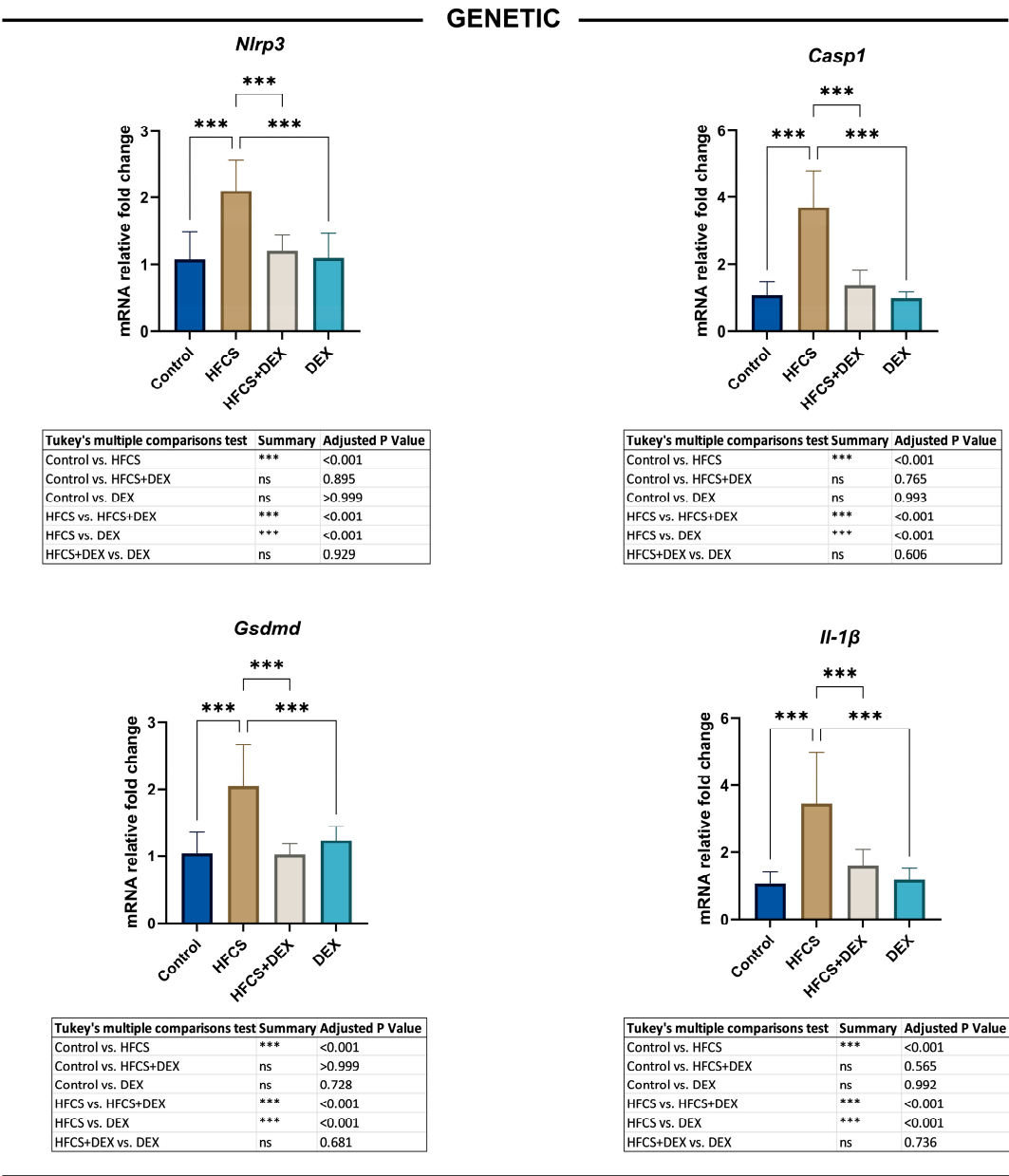


Figure 5. Gene expression analysis of inflammasome and pyroptosis-related markers in liver tissues. Relative mRNA expression levels of *Nlrp3*, *Casp1*, gasdermin D (*Gsdmd*), and interleukin-1β (*Il-1β*)

were significantly increased in the HFCS group compared to the control group, indicating activation of inflammasome signaling and pyroptotic pathways. DEX treatment (HFCS+DEX group) significantly downregulated the expression of these genes, demonstrating its modulatory effect on inflammatory and cell death pathways. The DEX-only group showed gene expression levels comparable to controls. Bar graphs represent mean $\pm$ SD of relative mRNA expression levels (fold change). Tables below the graphs present the adjusted p values obtained using one-way ANOVA followed by Tukey's multiple-comparisons test. Statistical significance is indicated as *** $p < 0.001$. **Abbreviations:** HFCS, high-fructose corn syrup; DEX, dexamethasone; *Nlrp3*, NOD-like receptor family pyrin domain containing 3; *Casp1*, caspase-1; *Gsdmd*, gasdermin D; *Il-1 β* , interleukin-1 beta.

2.4. Biochemical Oxidative Stress Analysis

Biochemical assessment of hepatic oxidative balance demonstrated no significant differences in total antioxidant status (TAS) among the control, HFCS, HFCS+DEX, and DEX groups (all adjusted $p > 0.05$). In contrast, total oxidant status (TOS) was significantly higher in the HFCS group than in the control group ($p < 0.001$) and the DEX group ($p = 0.017$). TOS was also significantly elevated in the HFCS+DEX group compared with the control group ($p = 0.006$). Although the mean TOS level was lower in the HFCS+DEX group than in the HFCS group, this difference did not reach statistical significance ($p = 0.276$). No significant differences were observed between the control and DEX groups ($p = 0.122$) or between the HFCS+DEX and DEX groups ($p = 0.541$). These findings indicate that HFCS exposure increased hepatic oxidant burden without producing a measurable change in total antioxidant capacity, whereas DEX treatment was associated with a nonsignificant downward trend in TOS under the present experimental conditions (Figure 5).

OXIDATIVE STRESS PARAMETERS

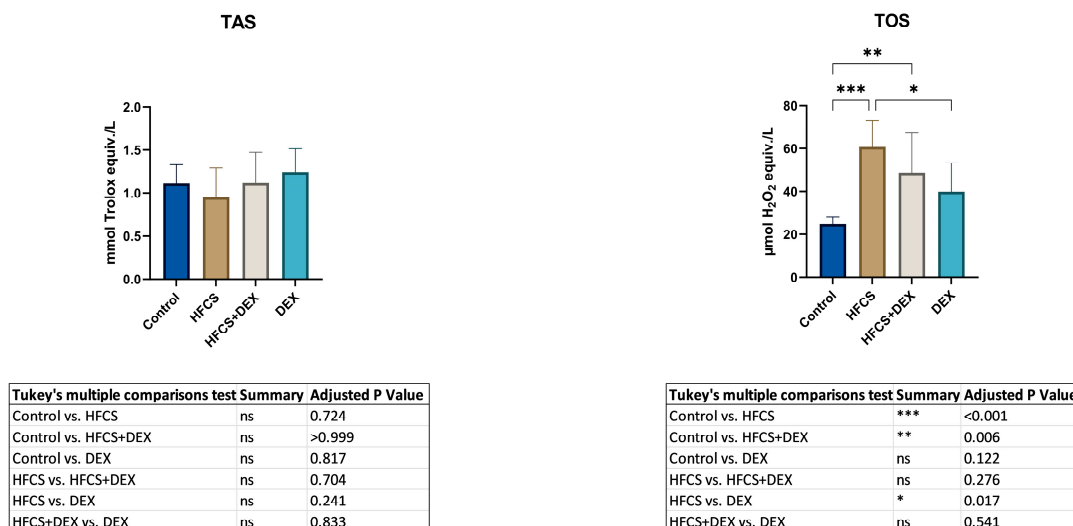


Figure 5. Total antioxidant status and total oxidant status in liver tissues across experimental groups. Total antioxidant status (TAS) did not differ significantly among the control, HFCS, HFCS+DEX, and DEX groups. Total oxidant status (TOS) was significantly increased in the HFCS group compared with the control and DEX groups. TOS was also significantly higher in the HFCS+DEX group than in the control group. Although TOS was numerically lower in the HFCS+DEX group than in the HFCS group, the difference was not statistically significant. Bars represent mean $\pm$ SD. Statistical comparisons were performed using one-way ANOVA followed by Tukey's multiple-comparisons test. Statistical significance is indicated as * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. **Abbreviations:** TAS, total antioxidant status; TOS, total oxidant status; HFCS, high-fructose corn syrup; DEX, dexamethasone.

3. Discussion

The present study demonstrated that HFCS exposure caused marked histopathological liver injury, increased hepatic oxidant burden and lipid peroxidation, enhanced apoptotic activity, and upregulated inflammasome- and pyroptosis-associated genes in young adult rats. The principal novel finding was that DEX treatment attenuated histopathological injury and significantly reduced MDA and Cas-3 immunoreactivity while downregulating *Nlrp3*, *Casp1*, *Gsdmd*, and *Il-1 β* mRNA expression. These findings indicate that the hepatoprotective effects of DEX were associated with attenuation of lipid peroxidation, apoptosis, and inflammasome- and pyroptosis-associated transcriptional signaling. However, because protein cleavage, cytokine maturation, and membrane-pore formation were not directly assessed, the present results do not definitively demonstrate the activation or inhibition of pyroptotic cell death.

The pediatric relevance of these findings deserves particular emphasis. Excessive intake of fructose-containing beverages and processed foods has been closely linked to pediatric MASLD, hepatic steatosis, dyslipidemia, and cardiometabolic risk, suggesting that fructose exposure may represent an early dietary driver of metabolic liver injury in children and adolescents. Pediatric MASLD studies have also implicated oxidative stress as an important contributor to disease development and progression, while experimental adolescent models indicate that fructose-rich diets may promote hepatic lipid accumulation, inflammatory responses, oxidative stress, and activation of the NLRP3 inflammasome pathway. Therefore, the use of young adult rats in the present study provides a developmentally relevant framework for evaluating early hepatic responses to HFCS exposure. Although direct pediatric data linking fructose exposure to pyroptosis remain limited, our findings support the concept that oxidative stress, apoptosis, and inflammasome-related pyroptotic signaling may act as interconnected mechanisms in early-life fructose-induced liver injury. In this context, DEX is also of translational interest because dextran and pantothenic acid derivatives have a long history of clinical use, particularly in pediatric dermatologic and mucosal barrier-supportive formulations; however, its systemic hepatoprotective role in pediatric metabolic liver disease remains unexplored and should be investigated cautiously in future translational studies [17–19].

Importantly, DEX treatment markedly attenuated all these pathological changes, suggesting that its hepatoprotective effects are mediated through a multi-target mechanism involving suppression of oxidative stress, inhibition of apoptosis, and modulation of inflammasome-driven pyroptosis. These findings align with recent evidence indicating that metabolic liver injury is not solely driven by lipid accumulation but also involves complex inflammatory and cell death pathways, particularly those regulated by the NLRP3 inflammasome [5,6,20,21].

Mechanistically, the findings of the present study support a sequential injury model in which HFCS-induced oxidative stress acts as an upstream trigger of inflammasome activation. Increased MDA immunoreactivity suggests enhanced lipid peroxidation and ROS generation, which are known to activate TXNIP-dependent *Nlrp3* signaling. Subsequent activation of *Casp1* and *Gsdmd* may promote pyroptotic hepatocyte death and *Il-1 β* release, thereby amplifying hepatic inflammation. The concurrent increase in PCNA expression likely reflects a compensatory regenerative response to ongoing hepatocellular loss. DEX appears to interrupt this injury cascade at multiple levels by reducing oxidative stress, limiting apoptotic signaling, and suppressing inflammasome-associated pyroptosis. Increased PCNA expression in the HFCS group should not be interpreted solely as beneficial proliferation, but rather as a compensatory regenerative response secondary to hepatocyte injury, apoptosis, and inflammatory damage.

The histopathological findings of the present study are consistent with previous experimental models demonstrating that excessive fructose consumption induces hepatic steatosis, inflammatory infiltration, and hepatocellular injury. In particular, the predominance of lipidosis and necrosis in centrilobular regions observed in our study is in agreement with earlier reports showing that this zone is more susceptible to metabolic and oxidative stress due to its lower oxygenation and higher exposure to toxic metabolites [22,23].

High-fructose intake has been shown to promote *de novo* lipogenesis, increase triglyceride accumulation, and disrupt hepatic metabolic balance, ultimately leading to steatosis and structural liver damage. Moreover, fructose-driven metabolic dysregulation contributes to the progression from simple steatosis to steatohepatitis by enhancing oxidative stress and inflammatory responses [24,25].

The observed increase in hyperemia, hemorrhage, and inflammatory cell infiltration in the HFCS group further supports the concept that metabolic liver injury is not solely driven by lipid accumulation but involves a complex interaction between metabolic overload and inflammatory signaling pathways. Importantly, the significant improvement in histopathological parameters following DEX treatment in our study is consistent with previous findings demonstrating that antioxidant and anti-inflammatory agents can effectively attenuate fructose-induced hepatic damage [9]. These findings suggest that DEX mitigates HFCS-induced liver injury not only by reducing lipid accumulation but also by suppressing inflammation and cellular damage, thereby contributing to the restoration of hepatic architecture.

Oxidative stress is a key driver of fructose-induced liver injury and plays a central role in the transition from simple steatosis to steatohepatitis. In the present study, the significant increase in MDA expression in the HFCS group clearly indicates enhanced lipid peroxidation and oxidative damage within hepatocytes. MDA is a well-established marker of oxidative stress, reflecting the extent of membrane lipid degradation caused by ROS. These findings are consistent with previous studies demonstrating that high-fructose intake increases ROS production, impairs antioxidant defense systems, and promotes oxidative injury in liver tissue [26,27]. The biochemical TAS and TOS findings provide complementary evidence regarding the oxidative imbalance induced by HFCS. HFCS exposure significantly increased hepatic TOS, whereas TAS remained comparable across all groups. This pattern suggests that oxidative stress in the present model was predominantly driven by enhanced oxidant generation rather than by a detectable depletion of the overall antioxidant reserve. A preserved TAS level does not necessarily indicate adequate protection against oxidative injury, because quantitatively maintained antioxidant systems may still be insufficient to neutralize excessive reactive oxygen species production. The concurrent increase in TOS and MDA immunoreactivity therefore supports the presence of a biologically relevant pro-oxidant state in HFCS-exposed liver tissue [14,27,28].

In the HFCS+DEX group, TOS was numerically lower than in the HFCS group; however, this reduction was not statistically significant, and TOS remained significantly higher than in the control group. Therefore, the TAS and TOS findings should not be interpreted as evidence that DEX completely restored global hepatic redox balance. Nevertheless, DEX treatment significantly reduced MDA immunoreactivity, indicating attenuation of lipid peroxidation at the tissue level. The apparent difference between the TOS and MDA results may be explained by the distinct biological information provided by these measurements. TOS represents a composite estimate of multiple oxidant molecules in tissue homogenates, whereas MDA immunostaining more specifically reflects lipid peroxidation and its cellular distribution within liver tissue. Differences in analytical sensitivity, biological variability, treatment duration, and the temporal kinetics of oxidant production and lipid damage may also have contributed to this divergence. Accordingly, DEX appeared to exert a more

evident protective effect on lipid peroxidation and structural tissue injury than on the total hepatic oxidant pool under the present experimental conditions [9,10].

In parallel with oxidative stress, we observed a marked increase in Cas-3 expression in the HFCS group, indicating activation of apoptotic pathways. Cas-3 is a key executioner caspase in apoptosis and is commonly upregulated in response to mitochondrial dysfunction and oxidative damage. The coexistence of elevated MDA and Cas-3 levels in our study suggests a strong link between oxidative stress and apoptosis in HFCS-induced hepatocellular injury. This relationship has been well documented, with ROS-mediated mitochondrial damage triggering caspase activation and subsequent hepatocyte apoptosis in metabolic liver disease models [29,30].

Importantly, DEX treatment significantly reduced MDA and Cas-3 expression and was associated with a nonsignificant downward trend in TOS, without significantly altering TAS. These findings support the antioxidant and anti-apoptotic effects of DEX but indicate that its influence on the overall hepatic oxidant–antioxidant balance was partial rather than complete. These findings are in line with previous reports showing that DEX enhances cellular antioxidant capacity, stabilizes mitochondrial function, and reduces ROS-mediated cellular injury. By attenuating oxidative stress, DEX likely interrupts the cascade leading to apoptosis, thereby preserving hepatocyte integrity [9,10].

Collectively, these results suggest that oxidative stress-induced apoptosis is a major contributor to HFCS-mediated liver injury and that DEX exerts its hepatoprotective effects, at least in part, through the suppression of ROS generation and inhibition of apoptosis.

A central finding of the present study is the marked activation of the NLRP3 inflammasome and its downstream pyroptotic signaling cascade in HFCS-induced liver injury. We observed significant upregulation of *Nlrp3*, *Casp1*, *Gsdmd*, and *Il-1 β* expression, indicating that inflammasome-mediated pyroptosis plays a pivotal role in hepatocellular damage in this model. The *Nlrp3* inflammasome is a cytosolic multiprotein complex that is activated in response to metabolic stress signals, including ROS, lipid accumulation, and mitochondrial dysfunction, all of which are characteristic features of fructose-induced liver injury [20,31].

Upon activation, *Nlrp3* promotes the cleavage and activation of *Casp1*, which subsequently processes pro-*Il-1 β* into its active form and induces the cleavage of *Gsdmd*. The N-terminal fragment of *Gsdmd* forms pores in the cell membrane, leading to cellular swelling, membrane rupture, and release of pro-inflammatory cytokines, a process defined as pyroptosis. This highly inflammatory form of programmed cell death amplifies hepatic inflammation and contributes to the progression of metabolic liver disease [7,32].

Recent studies have demonstrated that *Nlrp3* inflammasome activation is closely associated with the development and progression of MASLD, where it links metabolic stress to innate immune activation and hepatocellular injury. Inhibition of this pathway has been shown to reduce hepatic inflammation, steatosis, and fibrosis, highlighting its importance as a therapeutic target [5,21].

Importantly, our findings show that DEX significantly downregulated the expression of *Nlrp3*, *Casp1*, *Gsdmd*, and *Il-1 β* , suggesting that its hepatoprotective effects are mediated, at least in part, through suppression of inflammasome activation and pyroptotic cell death. Although the exact molecular mechanisms underlying this effect remain to be fully elucidated, it is likely that DEX interferes with upstream triggers of inflammasome activation, such as oxidative stress and mitochondrial dysfunction. By attenuating these signals, DEX may prevent the initiation of the inflammasome cascade and thereby reduce pyroptosis-driven inflammation.

These results position DEX as a potential modulator of inflammasome activity and highlight pyroptosis as a key therapeutic target in fructose-induced liver injury.

DEX has been widely recognized for its antioxidant, anti-inflammatory, and cytoprotective properties in various experimental models of tissue injury. Previous studies have demonstrated that DEX reduces oxidative stress, limits lipid peroxidation, and attenuates inflammatory responses in different organ systems, including liver, kidney, and intestinal tissues. Moreover, DEX has been shown to modulate apoptotic pathways by regulating key mediators such as cas-3, caspase-9, and BAX/BCL2 balance, thereby promoting cell survival and tissue repair [9,12].

However, the potential role of DEX in regulating inflammasome activation and pyroptosis has not been previously elucidated. To the best of our knowledge, this is the first study to demonstrate that DEX suppresses the *Nlrp3–Casp1–Gsdmd* axis in a model of fructose-induced liver injury. This finding represents a significant extension of the known mechanisms of DEX, shifting its role from a general antioxidant agent to a targeted modulator of inflammatory cell death pathways.

The novelty of our study lies in integrating histopathological, immunohistochemical, and molecular data to demonstrate that DEX exerts a multi-layered protective effect by simultaneously attenuating oxidative stress, apoptosis, and pyroptosis. While previous studies have primarily focused on the antioxidant and anti-apoptotic effects of DEX, our results highlight its ability to regulate upstream inflammatory signaling pathways, particularly the inflammasome complex. This is of particular importance, as pyroptosis has recently emerged as a key driver of inflammation and disease progression in metabolic liver disorders [21,33].

From a translational perspective, these findings suggest that DEX may offer a novel therapeutic strategy targeting both metabolic and inflammatory components of liver injury. Unlike conventional approaches that focus on downstream cytokine inhibition, DEX appears to act at an earlier stage by modulating fundamental cellular stress and death pathways. This dual-action mechanism may provide a broader and more effective therapeutic benefit, particularly in complex diseases such as metabolic-associated fatty liver disease.

In this context, our findings may have particular relevance for pediatric hepatology, as they suggest that inflammasome activation and pyroptosis may play a critical role in early-stage liver injury induced by dietary factors such as HFCS. The demonstration that DEX effectively attenuates these pathways in a young adult animal model raises the possibility that it may serve as a potential therapeutic or preventive strategy in pediatric populations at risk of metabolic liver disease.

Moreover, considering that pediatric patients often present with more aggressive disease progression and limited therapeutic options, interventions targeting early pathogenic mechanisms, such as oxidative stress and inflammasome activation, may offer significant clinical benefit. Therefore, the use of a young adult animal model not only strengthens the translational relevance of our findings but also highlights the importance of investigating age-specific mechanisms in metabolic liver disease.

This study has several limitations that should be acknowledged. First, the experimental design is based on an animal model, which may limit the direct generalizability of the findings to human disease. Although the use of young adult rats increases the translational relevance to pediatric populations, species-specific differences in metabolism and immune responses should be considered. Second, while we demonstrated significant modulation of inflammasome-related gene expression, protein-level validation (e.g., Western blot or ELISA) was not performed, which may limit the mechanistic depth of the findings. Additionally, other relevant pathways involved in metabolic liver disease, such as insulin resistance, gut–liver axis alterations, and microbiota changes [34,35], were beyond the scope of the present study. Serum biochemical markers were not measured in the present study. Therefore, the tissue-level findings could not be correlated with circulating markers of hep-

atocellular injury or cholestasis, such as ALT, AST, ALP, and GGT, or with indicators related to hepatic synthetic and excretory function, including albumin and bilirubin. Consequently, the present results demonstrate histopathological and molecular attenuation of hepatic injury but do not establish an improvement in biochemical liver function. Future studies should combine tissue analyses with a comprehensive serum liver biochemical panel.

Despite these limitations, our study provides comprehensive histopathological, immunohistochemical, and molecular evidence supporting the role of oxidative stress, apoptosis, and inflammasome-mediated pyroptosis in HFCS-induced liver injury. Future studies should aim to validate these findings in larger animal cohorts and clinical settings, incorporate protein-level analyses, and explore the long-term effects of DEX on fibrosis and disease progression. Furthermore, investigating the interaction between inflammasome activation and gut microbiota, as well as assessing combination therapies targeting multiple pathogenic pathways, may provide deeper insight into the management of metabolic liver disease [36]. Although the present study demonstrated robust transcriptional suppression of *Nlrp3*, *Casp1*, *Gsdmd*, and *Il-1 β* , future studies incorporating cleaved GSDMD, cleaved caspase-1, IL-18, and protein-level validation are required to definitively confirm pyroptotic cell death.

In conclusion, HFCS exposure caused histopathological liver injury, increased hepatic oxidant burden and lipid peroxidation, enhanced apoptotic activity, and upregulated *Nlrp3*-, *Casp1*-, *Gsdmd*-, and *Il-1 β* -related transcriptional signaling in young adult rats. DEX treatment attenuated hepatic structural injury, reduced MDA and Cas-3 immunoreactivity, and downregulated inflammasome- and pyroptosis-associated gene expression. These findings support a potential multi-target hepatoprotective effect of DEX in this experimental model. However, the absence of protein-level validation and direct assessment of pyroptotic cell death requires cautious interpretation. Further mechanistic, age-comparative, and translational studies are needed before the clinical relevance of systemic DEX treatment can be determined.

4. Materials and Methods

4.1. Drugs and Chemicals

Dexpanthenol was obtained from a commercial pharmaceutical source (Bepanthen[®], Bayer, Istanbul, Türkiye). HFCS-55 solution containing 55% fructose and 41% dextrose was used to establish the fructose-induced metabolic injury model [37]. Ketamine hydrochloride (Ketasol 10%, Richter Pharma, Wels, Austria) and xylazine hydrochloride (Rompun 2%, Bayer, Leverkusen, Germany) were used for anesthesia. Formalin, hematoxylin–eosin staining solutions, and analytical-grade laboratory consumables were purchased from standard commercial suppliers. Primary antibodies used for immunohistochemical analysis included anti-caspase-3 p12 antibody (clone EPR16888, ab179517), anti-malondialdehyde (MDA) antibody (clone 11E3, ab243066), and anti-proliferating cell nuclear antigen (PCNA) antibody (clone EPR3821, ab92552) (Abcam, Cambridge, UK). Immunohistochemical detection was performed using the Mouse and Rabbit Specific HRP/DAB IHC Detection Kit–micro-polymer (ab236466) and 3,3'-diaminobenzidine (DAB) chromogen (Abcam, Cambridge, UK). RNA isolation and RT-qPCR reagents included the GeneAll RiboEx[™] RNA Isolation Kit (GeneAll Biotechnology, Seoul, Republic of Korea), A.B.T.[™] cDNA Synthesis Kit, and A.B.T.[™] 2X qPCR SYBR-Green MasterMix (Atlas Biotechnology, Ankara, Türkiye).

4.2. Animals and Experimental Design

A total of 32 young adult (8-week-old) male Wistar albino rats (weighing 200–250 g) were obtained from the Süleyman Demirel University Experimental Animal Research Center. The animals were approximately 16 weeks old at the time of euthanasia. Rats

at approximately 8–9 weeks of age are generally considered to be transitioning from late adolescence to early adulthood, although developmental classifications may vary according to strain, sex, and the physiological outcome under investigation. Accordingly, the animals in the present study are referred to as young adult rats rather than juvenile or pediatric-equivalent animals. This age was selected to investigate HFCS-induced hepatic injury and the response to dexpanthenol in a young-adult organism while limiting potential confounding from advanced age-related metabolic and hepatic alterations [38,39]. Animals were housed under standard laboratory conditions (12 h light/dark cycle, temperature 22 ± 2 °C, humidity 50–60%) with free access to standard pellet diet and water. From the end of week 4 until the end of the experiment, they received physiological saline intraperitoneally once daily to provide injection-related stress comparable to the treatment groups. Rats in the HFCS group received freshly prepared 20% HFCS-55 solution in drinking water for 8 weeks and were administered physiological saline intraperitoneally once daily from the end of week 4. Rats in the HFCS+DEX group received freshly prepared 20% HFCS-55 solution in drinking water for 8 weeks and were treated with dexpanthenol at a dose of 500 mg/kg/day intraperitoneally once daily from the end of week 4 until the end of week 8. The selected dose was based on previous experimental studies demonstrating antioxidant, anti-inflammatory, and cytoprotective effects without evidence of systemic toxicity [9,40,41]. Rats in the DEX group received standard drinking water for 8 weeks and were administered dexpanthenol at a dose of 500 mg/kg/day intraperitoneally once daily from the end of week 4 until the end of week 8. The 20% HFCS-55 solution was prepared by mixing 200 mL HFCS-55 with 800 mL drinking water until homogeneous. The solution was prepared fresh daily and placed equally into the drinking bottles of the HFCS-treated groups. Body weights were measured weekly to adjust dexpanthenol dose and saline volume. Water intake was recorded daily, and food intake was measured every two days. All experimental procedures were approved by the Süleyman Demirel University Animal Ethics Committee (Approval No: 15/425, Date: 12 December 2024) and conducted in accordance with ARRIVE 2.0 guidelines. Animals were randomly assigned into four groups ($n = 8$ per group):

Control group: Rats received standard drinking water throughout the experimental period. From the end of the fourth week until the end of the study, animals were administered physiological saline intraperitoneally (i.p.) once daily.

HFCS group: Rats in the HFCS group received a freshly prepared 20% (v/v) HFCS-55 solution in drinking water for 8 weeks. The HFCS exposure protocol was adapted from a previously described high-fructose dietary model [42]. From the end of week 4 until the end of week 8, the animals were administered 0.5–1 mL of physiological saline intraperitoneally once daily to reproduce the injection-related stress applied to the DEX-treated groups.

HFCS+DEX group: Rats were administered 20% HFCS in drinking water for 8 weeks. Beginning at the end of week 4, animals received DEX (500 mg/kg/day, i.p.) once daily and treatment was continued until the end of week 8.

DEX group: Rats received standard drinking water throughout the 8-week experimental period. From the end of week 4 until the end of week 8, the animals were administered DEX at a dose of 500 mg/kg/day intraperitoneally once daily.

Successful induction of the HFCS-associated hepatic injury model was evaluated at the end of the 8-week experimental period by comparing the HFCS group with the control group. The primary criteria were the presence of characteristic hepatic histopathological alterations, including lipidosis, inflammatory cell infiltration, hepatocellular ballooning, vascular congestion, hemorrhage, and necrotic changes, together with increased semi-quantitative histopathological scores.

At 24 h after the last administration, all animals were anesthetized with ketamine hydrochloride at 80–90 mg/kg and xylazine hydrochloride at 8–10 mg/kg, administered intraperitoneally, and euthanized by exsanguination under deep anesthesia. Liver tissues were rapidly excised, rinsed with cold saline, and divided for histopathological, immunohistochemical, and molecular analyses.

4.3. Histopathological Analysis

Liver tissue samples were fixed in 10% neutral buffered formalin for 24–48 h, processed using a standard tissue processor, and embedded in paraffin. Sections of 5 µm thickness were obtained using a rotary microtome and mounted on glass slides.

Sections were stained with hematoxylin and eosin (H&E) and examined under a light microscope (Olympus CX21, Tokyo, Japan). Histopathological evaluation included hyperemia, hemorrhage, inflammatory cell infiltration, necrosis, and lipidosis. Each feature was scored independently on a semi-quantitative scale ranging from 0 to 3 using a histopathological scoring framework adapted from Veteläinen et al. [43]. The operational definitions and severity categories applied in the present study are provided in Supplementary Table S1.

All slides were examined in a blinded manner by a qualified pathologist from an independent institution. For each marker, ten non-overlapping fields were randomly selected from each of the three sections obtained from each animal and evaluated at ×40 objective magnification. The field-level measurements were averaged to generate a single animal-level value. The percentage of positively stained cells was quantified using ImageJ software (National Institutes of Health, Bethesda, MD, USA; version 1.48). Measurements obtained from the evaluated fields were averaged to generate a single value for each animal, and the individual animal was considered the experimental unit for statistical analysis. Representative microphotographs were captured using the Database Manual CellSens Life Science Imaging Software System (Olympus Co., Tokyo, Japan).

4.4. Immunohistochemical Analysis

For immunohistochemical analysis, three sections were obtained from each paraffin-embedded liver block and mounted onto poly-L-lysine-coated slides to enhance tissue adherence. The HRP/DAB detection system was used to evaluate the expression of Cas-3, MDA, and PCNA. All primary antibodies were applied at a 1:100 dilution and incubated for 60 min at room temperature.

After primary antibody incubation, sections were treated with the appropriate secondary antibody and the HRP/DAB detection system according to the manufacturer's instructions. Negative controls were prepared by replacing the primary antibody with antigen dilution solution to ensure staining specificity.

All slides were examined by a qualified pathologist who was blinded to the experimental group allocation. For each marker, ten non-overlapping fields were randomly selected from each of the three sections obtained from each animal and evaluated at ×40 objective magnification. The percentage of positively stained cells was quantified using ImageJ software (National Institutes of Health, Bethesda, MD, USA; version 1.48). Field-level measurements were averaged to generate a single value for each animal, and the individual animal was considered the experimental unit for statistical analysis. Representative microphotographs were captured using the CellSens Life Science Imaging Software System (Olympus Co., Tokyo, Japan).

4.5. Reverse Transcription Quantitative Polymerase Chain Reaction (RT-qPCR) Analysis

RNA isolation from homogenized liver tissues was performed using a commercial RNA isolation kit according to the manufacturer's protocol. RNA concentration and purity were assessed using a BioSpec-nano spectrophotometer (Shimadzu Ltd., Kyoto, Japan).

Complementary DNA was synthesized from 1 µg of total RNA using a commercial cDNA synthesis kit and a Bio-Rad T100 Thermal Cycler (Bio-Rad, Hercules, CA, USA). Primer sequences were designed based on specific mRNA sequences obtained from the NCBI database and are listed in Table 1.

Table 1. Primary sequences, product size and accession numbers of genes.

Genes	Primer Sequence	Product Size	Accession Number
<i>Gapdh</i> (HouseKeeping)	F: AGTGCCAGCCTCGTCTCATA R: GATGGTGTATGGGTTTCCCGT	248 bp	NM_017008.4
<i>Nlrp3</i>	F: TCTCTGCATGCCGTATCTGG R: ACGGCGTTAGCAGAAATCCA	295 bp	NM_001191642.1
<i>Casp1</i>	F: GACCGAGTGGTTCCCTCAAG R: GACGTGTACGAGTGGGTGTT	108 bp	NM_012762.3
<i>Gsdmd</i>	F: GGGTGAAGATCGTGGTGAGC R: CGGCATGATCCAGAGTAGGG	75 bp	NM_001400994.1
<i>Il-1β</i>	F: TTGAGTCTGCACAGTTCCCC R: GTCCTGGGAAGGCATTAGG	161 bp	NM_031512.2

F: Forward, R: Reverse, *Gapdh*: Glyceraldehyde 3-phosphate dehydrogenase, *Nlrp3*: NLR family pyrin domain containing 3, *Casp1*: Caspase-1, *Gsdmd*: Gasdermin D, *Il-1β*: Interleukin-1 beta.

Gene expression levels were analyzed using an SYBR Green-based qPCR system. The reaction mixture was prepared in a final volume of 20 µL, and each sample was analyzed in triplicate using the Bio-Rad CFX Opus 96 real-time PCR system (Bio-Rad, Hercules, CA, USA). PCR cycling conditions were as follows: initial denaturation at 94 °C for 10 min, followed by 40 cycles of denaturation at 95 °C for 15 s and annealing/extension at 57 °C for 30 s. Relative mRNA expression levels were calculated using the $2^{-\Delta\Delta C_t}$ method after normalization to GAPDH.

4.6. Biochemical Assessment of Oxidative Stress

Liver tissue samples were homogenized in phosphate-buffered saline (PBS; 10 mM sodium phosphate, pH 7.4) at a ratio of 1:9 (*w/v*) using a tissue homogenizer (IKA Ultra Turrax T25, Janke & Kunkel, Staufen, Germany) under cold conditions. The homogenates were centrifuged at 10,000 rpm for 10 min at 4 °C using a refrigerated centrifuge (Nüve NF 1200R, Nüve, Ankara, Türkiye), and the resulting supernatants were collected for biochemical analyses.

Total antioxidant status (TAS) and total oxidant status (TOS) were determined in liver tissue homogenates using commercially available colorimetric assay kits (Elabscience, Wuhan, China; Cat. No: E-BC-K801-M and E-BC-K802-M) according to the manufacturer's instructions. Absorbance measurements were performed using a microplate reader (MS4 MaxiRead96 Microplate Absorbance Reader, Maxilab Biotechnology, Istanbul, Türkiye), and concentrations were calculated using standard calibration curves. TOS was selected as a composite measure of the cumulative oxidizing capacity of the liver homogenates and was expressed as H₂O₂ equivalents. It was not intended to identify a particular reactive oxygen species. TAS was used as an aggregate measure of antioxidant capacity, whereas MDA immunohistochemistry provided complementary information regarding lipid peroxidation and its distribution within hepatic tissue. Accordingly, these measurements were interpreted as indices of global oxidant burden, antioxidant capacity, and lipid oxidative damage rather than as direct measurements of specific reactive oxygen species.

TAS values were expressed as mmol Trolox equivalent/L, whereas TOS values were expressed as µmol H₂O₂ equivalent/L. These parameters were used to evaluate the overall oxidative stress status of hepatic tissue.

A schematic overview of the experimental design, including animal grouping, treatment procedures, and analytical methods, is presented in the Graphical Abstract.

4.7. Statistical Analysis

All statistical analyses were performed using GraphPad Prism software (version 10.1). The Shapiro–Wilk test was first applied to assess the normality of the data distribution. As the results indicated a normal distribution ($p > 0.05$), a one-way analysis of variance (ANOVA) was subsequently conducted to compare differences among the groups. To identify specific pairwise differences, Tukey’s post hoc multiple comparison test was applied. Statistical significance was set at $p < 0.05$. All results are presented as mean $\pm$ standard deviation (SD).

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/ijms27156828/s1>.

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