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Hepatoprotective effects of cinnamaldehyde against high-fat diet-induced liver damage

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Abstract

Objective: Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disorder worldwide, affecting 30–40% of adults. High-fat diets contribute significantly to NAFLD by promoting hepatic lipid accumulation, oxidative stress, and inflammation. In this context, cinnamaldehyde (CNMA) has emerged as a promising hepatoprotective agent due to its antioxidant, anti-inflammatory, and lipid-regulatory properties.

Materials and Methods: Male Wistar rats were randomly assigned to four groups (n=6 per group): (A) Control; (B) HFD; (C) Control+CNMA; and (D) HFD+CNMA. CNMA was administered orally at 20 mg/kg body weight for 16 weeks simultaneously with HFD. At the end of the study, rats were fasted for 12–14 hr and anesthetized with sodium pentobarbital (60 mg/kg, intraperitoneal) for serum, liver, and visceral adipose tissues collection. Biochemical analyses included serum liver enzymes, lipid profiles, hepatic triglyceride levels, and oxidative stress markers (nitric oxide metabolites; NOx, and malondialdehyde; MDA). Histopathological evaluation was performed on H&E (Hematoxylin and Eosin)-stained liver sections.

Results: HFD feeding induced significant hepatic injury and metabolic dysfunction in rats, characterized by elevated AST (Aspartate aminotransferase) and ALT (Alanine aminotransferase) levels, increased liver and fat pad weights, and enhanced oxidative stress. CNMA treatment significantly reduced these parameters, resulting in lower serum liver enzymes, decreased hepatic triglyceride content, reduced adiposity (notably mesenteric fat), and ameliorated oxidative stress. Histopathological findings confirmed a reduction in micro- and macrovesicular steatosis with CNMA.

Conclusion: CNMA significantly protected against HFD-induced hepatic injury by reducing serum AST and ALT, hepatic triglycerides, visceral adiposity, and oxidative stress and inflammatory markers, as confirmed by histopathology. It suggests the therapeutic potential of CNMA for NAFLD and related metabolic disorders.

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Introduction

Non-alcoholic fatty liver disease (NAFLD) affects 30–40% of adults globally, positioning it as the most prevalent chronic liver disorder (Teng et al. 2023). NAFLD spans a spectrum from simple steatosis to non-alcoholic steatohepatitis (NASH), which may progress to fibrosis, cirrhosis, and hepatocellular carcinoma (Masuoka and Chalasani 2013; Singh et al. 2014). In the United States, it is projected that NAFLD will affect nearly 101 million individuals by 2030 (Ortiz-Lopez et al. 2012; Calabrese et al. 2022). Thereby, there is an urgent need for innovative therapeutic strategies to mitigate liver damage and improve metabolic outcomes.

The mechanisms underlying NAFLD are multifactorial, with high-fat diets (HFD) playing a pivotal role in its development by promoting hepatic lipid accumulation, oxidative stress, and inflammation (Cobbina and Akhlaghi 2017; Arroyave-Ospina et al. 2021). Excessive dietary fats lead to lipid overload in hepatocytes, which in turn, elevates reactive species such as NOx (Nitrate + Nitrite) and malondialdehyde (MDA) — key indicators of oxidative stress and cellular injury (Arroyave-Ospina et al. 2021). Understanding these mechanisms is critical for the development of effective interventions to reverse NAFLD pathology.

Cinnamaldehyde (CNMA; C_9H_8O), an active compound derived from cinnamon, has emerged as a promising candidate for hepatoprotection due to its antioxidant, anti-inflammatory, and lipid-modulatory effects (Ahmad et al. 2019; Tang et al. 2022). Previous research suggests that CNMA enhances insulin sensitivity and reduces hepatic lipid accumulation, potentially mitigating the adverse metabolic impacts of HFDs (Ahmad et al. 2019).

In this study, we aimed to evaluate the hepatoprotective effects of CNMA on liver function, lipid metabolism, oxidative stress, and histological alterations in a rat model of NAFLD induced by HFD.

Materials and Methods

Dietary regimens

This study utilized two distinct dietary protocols: low-fat (LFD) and high-fat (HFD). The LFD, with an energy density of 2.8 kcal/g, consisted of 20% protein, 1% fat, and 79% carbohydrate by weight, with 2.7% of energy derived from soybean oil. In contrast, the HFD, with a higher energy density of 4.1 kcal/g, comprised 18% protein, 25% fat, and 57% carbohydrate by weight, with 40% of energy derived from tail-fat. Both diets were provided by Javaneh-Khorasan Company, Iran (catalogue numbers 47037 and 47036, in LFD and HFD, respectively). Fatty acid composition, analysed via gas chromatography, revealed that the LFD contained 61.5% polyunsaturated fatty acid (PUFA), 16.1% saturated fatty acids (SFA), and 22.0% monounsaturated fatty acid (MUFA). In contrast, the HFD contained 3.45% PUFA, 44.9% SFA, and 51.64% MUFA (Farrokhfall et al. 2014).

Study protocol and animal subjects

A total of 24 male Wistar rats (12 weeks old, weighing 220–250 g) were obtained from the Pasteur Institute in Tehran, Iran. All procedures followed standard ethical guidelines and received approval from the local ethics committee of the Birjand University of Medical Sciences (IR.BUMS.AEC.1404.004), following NIH guidelines for animal care (NIH Publications No. 8023, revised 1978).

The rats were housed individually in the cages (45, 25, 15 cm) under controlled conditions (12-hr light and dark cycle, temperature at 24°C, and humidity control) with *ad libitum* access to food and water.

Rats were randomly assigned to four experimental groups (n=6 per group):

1. Control (LFD): Received a low-fat diet,
2. Control+CNMA: Received CNMA in addition to the LFD,

3. HFD: Received high-fat diet,
4. HFD+CNMA: Received CNMA in addition to the HFD.

Due to the small sample size, randomization based solely on chance could result in unbalanced group characteristics. To minimize this risk, stratified randomization was performed using baseline body weight as a covariate, given the study's focus on dietary intervention (Verhave et al. 2024). Blinding was implemented throughout the experiment, including during treatment administration, animal follow-up, and outcome assessment. Importantly, the investigator responsible for histopathological evaluations was blinded to group assignments to minimize observer bias (Karp et al. 2022).

The study spanned 16 weeks, during which the respective diet and treatments were administered. CNMA (W228613, Sigma-Aldrich, Shanghai, China; purity ≥95%) was administered daily for 16 weeks via gavage at a dose of 20 mg/kg, dissolved in corn oil. As a control for the gavage-related stress, rats not receiving CNMA were given corn oil.

Insulin tolerance test (ITT)

In the 15th week, an intraperitoneal insulin test (ITT) was performed on non-fasting rats. Blood samples (100 µl) were collected from the tail at the baseline (0 min) and 15 and 30-min after an intraperitoneal injection of regular human insulin (1 unit/kg in 1 ml saline) to assess triglyceride (TAG) levels (Catalogue Number: 10-1250-10, Mercodia Uppsala, Sweden).

At the end of week 16, following a 12–to 14-hr fast, rats were anesthetized with sodium pentobarbital (60 mg/kg), and blood was collected via cardiac puncture. The serum was separated by centrifugation (2000 rpm for 20 min) and stored at −20°C for further analysis. Cold saline (0.9% NaCl) was used for cardiac perfusion, followed by a midline laparotomy to harvest organs. The liver and visceral white adipose tissue (WAT) fat pads, including retroperitoneal, epididymal, and mesenteric depots, were isolated, weighed, and stored at -80°C until NOx and MDA measurements. A liver sample was also fixed in 10% formalin for histological analysis.

Measurement of triglycerides in liver tissue

Liver triglyceride levels were determined in three steps: 1. Fat extraction from liver tissue using the Folch method (Folch et al. 1957) (100 mg of liver tissue was homogenized with 1.2 ml of a chloroform/methanol mixture (2:1, v/v) and filtered through a specialized filter. The extract was left at room temperature until the solvent evaporated. 2. Triglyceride separation using the Danno method (Danno et al. 1992) (The fat extract was redissolved in 120 µl of tert-butyl alcohol and 80 µl of a Triton X-100/methyl alcohol mixture (1:1, v/v)), and 3. Quantification using a triglyceride standard kit from Zistchem Co. (A 10 µl aliquot of this solution was used for triglyceride measurement against a blank solution, which did not contain the triglyceride-extracting solvents). Triglyceride levels were calculated and reported as milligrams per 100 grams of liver tissue.

Protein concentration in the liver extract was measured using a protein assay kit based on the Bradford method (Bradford 1976). For liver extract preparation, 100 mg of liver tissue was homogenized in 1000 µl of phosphate-buffered saline (pH≈7.4) using a Micra D-1 homogenizer (Germany) while maintained in an ice-water bath, then the supernatant was separated by centrifuging (10000 g, for 15 min).

Biochemical assays

Serum analysis

Serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), total cholesterol (TC), and triglyceride were measured using

standard laboratory techniques provided by Zistchem Co (Iran).

Liver MDA assay

Liver MDA levels were determined using a thiobarbituric acid (TBA) reaction (Mihara and Uchiyama 1978). Briefly, 100 µl serum was combined with a reaction mixture containing 10% (v/v) trichloroacetic acid and 0.67% (w/v) TBA reagent in capped plastic tubes, then incubated in a boiling water bath for 45 min. After cooling to room temperature, 200 µl of *n*-butanol was added, and the samples were vortexed. Following centrifugation, the upper butanol layer was isolated, and its absorbance was measured at 532 nm using a spectrophotometer. MDA concentrations were calculated from a standard curve prepared with 1,1,2,3-tetraethoxypropane (1–20 µmol/L). Each measurement was performed in triplicate.

Liver NOx assay

As described earlier by Nakhaee et al. (Nakhaee et al. 2021), the Griess reaction was utilized to assess NOx concentrations in the liver. Briefly, 100 mg of liver tissue was homogenized in 1000 µl of phosphate-buffered saline (pH≈7.4) using a Micra D-1 homogenizer (Germany) while maintained in an ice-water bath. The resulting tissue homogenates underwent deproteinization using 90% ethanol, followed by vigorous shaking and subsequent centrifugation at 10,000 g for 15 min. To assay NOx levels, deproteinized serum or tissue extracts were combined with an equal volume of vanadium chloride (0.8% saturated solution in 1 M HCl) in a microplate. Subsequently, the reaction was initiated by adding the Griess reagent, prepared by mixing 0.2% sulfanilamide in 5% HCl and 0.1% NEDD (N-(1-naphthyl) ethylenediamine dihydrochloride) in distilled water, at a ratio of half the sample volume. Absorbance was recorded at 540 nm after incubating at 37° C for 30 min. NOx concentrations were extrapolated from a calibration curve generated with potassium nitrate (0–80 µmol).

Histopathological evaluation

Liver tissue samples were fixed in 10% neutral buffered formalin solution. Subsequently, the fixed specimens underwent routine histological processing, including dehydration through an ascending alcohol gradient, clearing with xylene, and embedding in paraffin blocks. Sections of 5-µm thickness were prepared from the tissue blocks and stained with hematoxylin and eosin (H&E).

Liver tissue sections were evaluated for steatosis severity according to the grading system established by (Liang et al. 2014). Steatosis was categorized into macrosteatosis and microsteatosis, with assessment based on hepatocellular hypertrophy (enlargement of hepatocytes) and intracellular lipid accumulation (classified as macro- or microvesicular steatosis). Each microscopic field was assigned a grade from 0 to 3 based on the percentage of affected hepatocytes:

Grade 0: <5% steatosis (normal)
Grade 1: Mild (6–33% involvement)
Grade 2: Moderate (34–66% involvement)
Grade 3: Severe (>66% involvement)

For quantitative analysis, 10 randomly selected microscopic fields per slide were graded, and the mean steatosis score was calculated (Hassanzadeh-Taheri et al. 2018; Dorranipour et al. 2024).

Statistical analysis

The normality of the data was verified using the Kolmogorov-Smirnov test. Data are presented as mean ± SEM, derived from triplicate experiments. ITT results were analysed using two-way ANOVA, while other data were assessed with one-way ANOVA in GraphPad Prism software (Version 9). *Post hoc* pairwise comparisons were performed using the Bonferroni test. The Kruskal-Wallis test was used to compare the pathological scores among the

studied groups. Statistical significance was set at $p<0.05$.

Results

Liver function tests and serum lipid profile

Serum levels of AST and ALT were measured to assess hepatic injury across all experimental groups. Rats in the HFD group exhibited significantly elevated AST and ALT levels compared to the control group ($p<0.05$ for AST, $p<0.001$ for ALT; Table 1).

CNMA administration in HFD-fed rats significantly reduced these enzyme levels, lowering AST to 51.67±31.57 U/L and ALT to 35.67±13.52 U/L ($p<0.05$ for AST, $p<0.001$ for ALT). In contrast, CNMA treatment in LFD rats did not produce significant changes in AST or ALT compared to the controls, suggesting no adverse hepatic effects in healthy animals. Additionally, serum TC and TAG levels did not differ significantly between experimental groups ($p>0.05$; Table 1).

Table 1. Serum level of lipid profile and liver function tests

Variable	Groups				p value
	Control Mean ± (SD)	Control+CNMA Mean ± (SD)	HFD Mean ± (SD)	HFD+CNMA Mean ± (SD)	
Plasma AST (U/L)	49.50±37.96	48.33±34.59	103.3±14.14*	51.67±31.57†	0.023
Plasma ALT (U/L)	39.00±28.89	45.67±34.60	93.83±13.47**	35.67±13.52††	0.0005
TAG (mg/dl)	102.00±8.42	101.90±19.97	107.50±22.20	103.50±13.27	0.93
TC (mg/dl)	82.50±7.96	81.33±8.47	89.00±12.25	86.17±10.68	0.68

CNMA: Cinnamaldehyde, HFD: High-Fat Diet, SD: Standard Deviation, AST: Aspartate Aminotransferase, ALT: Alanine Aminotransferase, TAG: Triglycerides, TC: Total Cholesterol. Statistical comparison among the groups was made using a one-way ANOVA followed by a Bonferroni *post-hoc* test; values are mean ± SD, n= 6 per group. *p<0.05 and **p<0.01, statistically significant differences between the Control and HFD; †p<0.05 and ††p<0.0001, statistically significant differences between the HFD+CNMA and HFD. Sampling was conducted on fasting.

Insulin tolerance test (ITT) and plasma triglyceride response

In the ITT, non-fasting plasma triglyceride levels at time zero (before insulin administration, 1 U/kg body weight) were significantly elevated in both the HFD (188.9±21.85 mg/dl, $p<0.001$) and HFD+CNMA (163.1±0.06 mg/dl, $p<0.05$) groups compared to the control group (118.7±0.14 mg/dl; Figure 1).

Following insulin administration, triglyceride concentrations declined across all groups ($p<0.05$). At 15 min post-injection, the HFD+CNMA group demonstrated a lower mean triglyceride level compared to the HFD group (120.7±4.99 mg/dl vs 146.4±22.99 mg/dl, respectively). By 30 min, the differences in plasma triglyceride levels among the groups were no longer statistically significant (Control: 91.3±6.05 mg/dl, CNMA: 103.4±5.03 mg/dl, HFD: 124.0±3.8 mg/dl, HFD+CNMA: 107.4±5.05 mg/dl; Figure 1).

Visceral adiposity and liver weight index

The relative weights of epididymal, retroperitoneal, and mesenteric fat pads (expressed as a percentage of body weight) were significantly elevated in the HFD group compared to the control group (Table 2). CNMA administration markedly reduced mesenteric fat accumulation in HFD-fed rats ($p<0.001$, Table 2).

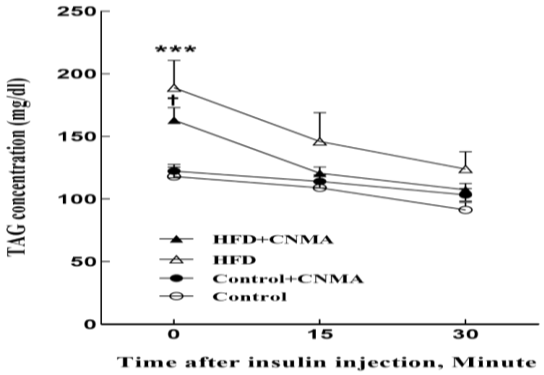

Figure 1. Plasma triglyceride changes during the Insulin tolerance test (ITT). Plasma triglyceride during ITT in the 4 groups. Data are presented as mean ± SEM (n=6 per group). $^{***}p<0.001$ significant difference between the HFD group and control, †p<0.05 HFD+CNMA vs HFD group. CNMA: Cinnamaldehyde, HFD: High-Fat Diet, TAG: Triglycerides.

Analysis of liver weight demonstrated a significant increase in the liver index (liver-to-body weight ratio) in the HFD group relative to controls ($p<0.001$, Figure 2a). CNMA administration in the HFD group significantly reduced this ratio ($p<0.05$, Figure 2a), although the liver index remained significantly higher than that of the control group ($p<0.01$, Figure 2a).

Hepatic triglyceride levels

Hepatic triglyceride content was significantly elevated in the HFD group compared to the controls ($p<0.001$). However, co-administration of CNMA with the HFD significantly reduced hepatic triglyceride levels ($p<0.05$, Figure 2b).

Specifically, the mean hepatic triglyceride concentration was 2.16±0.29 mg per 100 mg tissue in the control group, which increased to 3.71±0.97 mg per 100 mg in the HFD group. CNMA treatment lowered this value to 2.06±0.34 mg per 100 mg of liver tissue (Figure 2b). All values are expressed as milligrams of triglyceride per 100 mg of hepatic tissue.

Table 2. Fat pad index (fat pad weight/body fat ×100) in experimental groups

Fat pad index	Groups			
	Control Mean ± (SD)	Control+CNMA Mean ± (SD)	HFD Mean ± (SD)	HFD+CNMA Mean ± (SD)
Epidydimal (% BW)	1.07±0.32	1.04±0.24	1.61±0.17*	1.24±0.35
Retroperitoneal (% BW)	0.80±0.36	0.81±0.32	1.48±0.42*	1.03±0.35
Mesentery (% BW)	0.68±0.14	0.66±0.16	1.22±0.49*	0.62±0.10††

CNMA: Cinnamaldehyde, HFD: High-Fat Diet, SD: Standard Deviation, BW: Body Weight. Statistical comparison among the groups was made using a one-way ANOVA followed by a Bonferroni *post-hoc* test; values are mean ± SD, n= 6 per group. *p<0.05 and ***p<0.001, statistically significant differences between the Control and HFD; ††p<0.01, statistically significant differences between the HFD+CNMA and HFD.

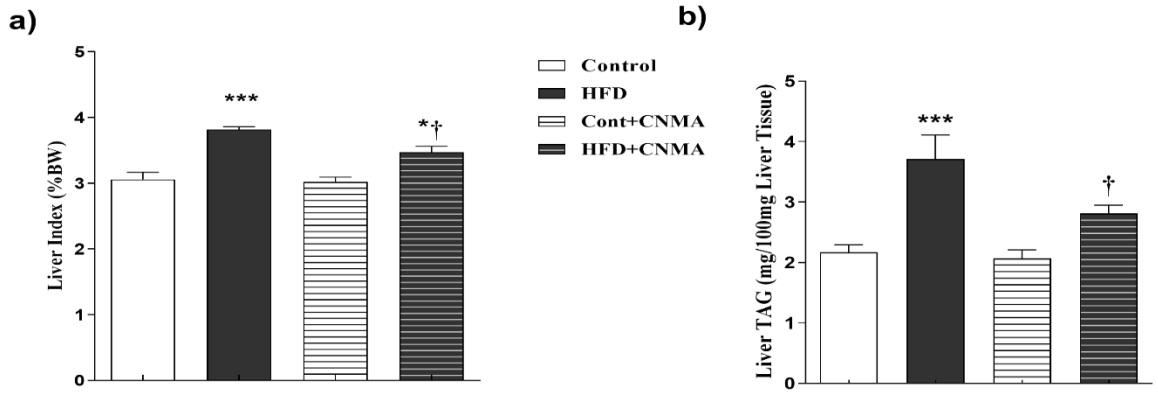

Figure 2. Comparison of liver index (liver weight to body weight ratio %, 2a) and liver triglyceride levels (2b) in experimental groups. Data are presented as mean ± SEM (n=6 per group). ***p<0.001 significant difference between the control and high-fat diet groups, †p<0.05 between the HFD and HFD+CNMA groups. The Kruskal-Wallis test was used for liver TAG compression among the experimental groups. CNMA: Cinnamaldehyde, HFD: High-Fat Diet, BW: Body Weight, TAG: Triglycerides.

Hepatic NOx levels

The hepatic NOx levels were significantly elevated in the HFD compared to the control rats (65.58±15.03 µmol/L vs 40.24±10.65 µmol/L in the HFD and Control groups, respectively, $p<0.005$). Treatment with CNMA resulted in a significant reduction in the hepatic NOx level (34.35±6.701 µmol/L, $p<0.001$). Specifically, CNMA administration significantly decreased NOx level to those comparable to the control rats (40.24±10.65 µmol/L vs 19.15±5.09 µmol/L in control and Control+CNMA groups, respectively, $p<0.01$; Figure 3).

The liver histopathological results

As illustrated in Figure 4a, the morphological analysis of liver samples

revealed distinct pathological alterations, including micro- and macrosteatosis, in the HFD group. In contrast, liver architecture remained normal in groups treated with CNMA. A quantitative assessment of the hepatic steatosis index demonstrated a significant increase in the HFD group compared to controls ($p<0.001$). Notably, CNMA administration markedly attenuated ($p<0.001$) this index relative to the HFD group (Figure 4b).

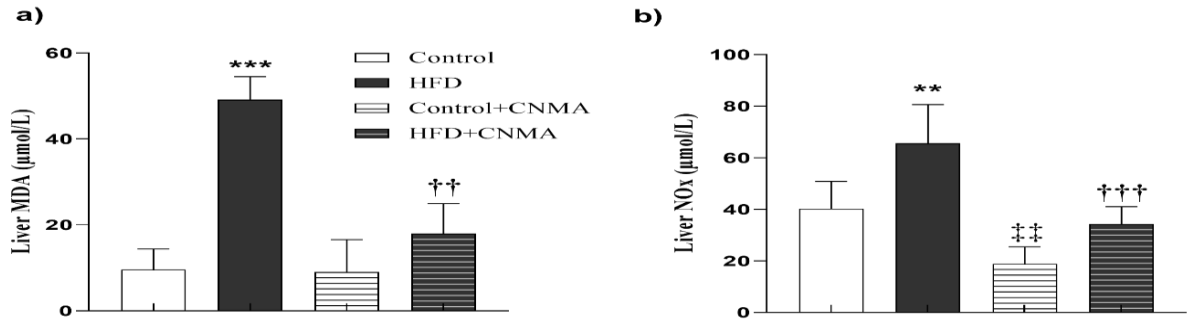

Figure 3. The hepatic oxidative/nitrosative stress in experimental groups. Malondialdehyde (3a) and NOx (3b) levels in the liver are presented as mean ± SEM (n=6 per group). $^{**}p<0.01$ and $^{***}p<0.001$ significant difference between the high-fat diet group and control, ††$p<0.01$ and †††$p<0.001$ HFD+CNMA vs HFD group, ‡‡$p<0.01$ between the control and control+CNMA groups. CNMA: Cinnamaldehyde, HFD: High-Fat Diet, MDA: Malondialdehyde, NOx: Nitrite + Nitrate

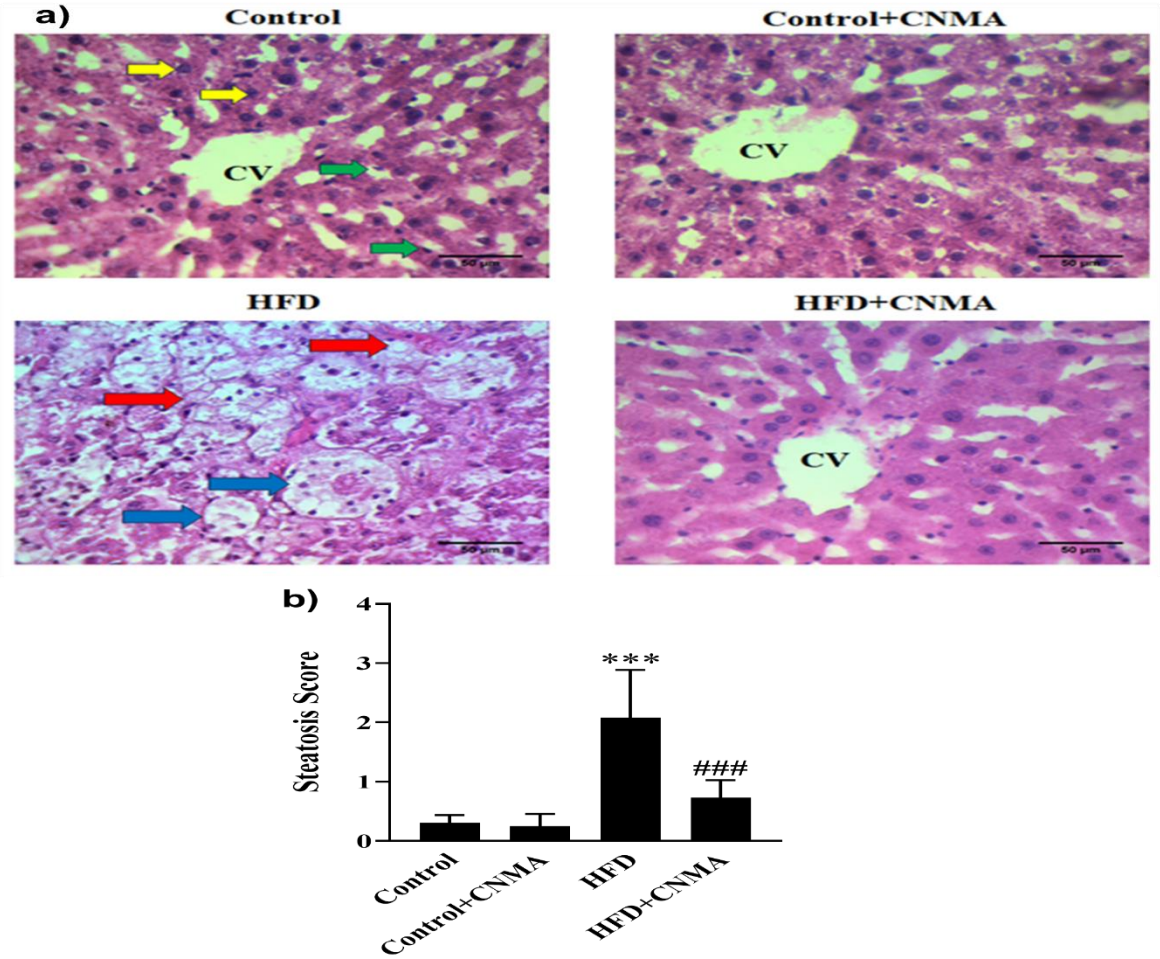

Figure 4. Effect of cinnamaldehyde (CNMA) on hepatic steatosis in high-fat diet (HFD)-fed rats. Representative H&E-stained photomicrographs (4a, 400× magnification; scale bar: 50 µm) show hepatocytes (yellow arrows), central vein (CV), and Kupffer cells (green arrows), microsteatosis (red arrows), and macrosteatosis (blue arrows). Quantitative analysis of hepatic steatosis in rats fed a low-fat diet (LFD) or high-fat diet (HFD) with or without cinnamaldehyde (CNMA) treatment (4b). Data are presented as mean ± SD. ***$p< 0.001$ vs. control; ###$p< 0.001$ vs. HFD.

Discussion

In this study, we investigated the metabolic and hepatic effects of CNMA in Wistar rats fed an HFD. CNMA administration significantly attenuated HFD-induced alterations, including reductions in serum liver enzymes (AST and ALT), hepatic triglyceride accumulation, intra-abdominal fat mass, and hepatic oxidative and inflammatory markers (NOx and MDA). These biochemical improvements were confirmed by histopathological findings. The consistency across biochemical, histological, and molecular endpoints suggests that CNMA exerts a multifactorial hepatoprotective effect, likely through modulation of lipid metabolism and attenuation of oxidative stress and inflammation.

In the present study, HFD-induced rats exhibited elevated hepatic triglyceride levels. Consistent with previous studies, CNMA consumption improved the lipid profile in HFD-fed rats, supporting its beneficial metabolic effects (Kannappan et al. 2006; Sheng et al. 2008; M. Safi 2015).

HMG-CoA, an essential enzyme in cholesterol synthesis, is upregulated by HFD and contributes to hepatic lipid accumulation and insulin resistance (M. Safi 2015; Matsumoto et al. 2016; Eraniappan et al. 2023). Previous studies suggest that cinnamon compounds, such as CNMA, can inhibit HMG-CoA reductase activity, thereby reducing cholesterol production and promoting lipid clearance in the liver (Ram et al. 2020; Liu et al. 2022). Additionally, HMG-CoA reductase activity is linked to several transcription factors and enzymes, such as sterol regulatory element-binding protein 1c (SREBP-1c), fatty acid synthase (FAS), and acetyl-CoA carboxylase (ACC), which regulate lipid metabolism (Eraniappan et al. 2023). There is evidence that cinnamon supplementation significantly influences the expression of genes related to lipogenesis and lipid storage in the liver, including *SREBP-1c*, *LXRs*, *ACLY*, and *FAS* (Lopes et al. 2015; Tuzcu et al. 2017; Kaur et al. 2019).

SREBP-1c promotes hepatic and adipose triglyceride accumulation by regulating lipogenic genes. However, cinnamon supplementation has been shown to reduce *SREBP-1c* expression, thereby decreasing lipogenesis and improving lipid metabolism (Lee et al. 2010; Han et al. 2015). Our findings align with those of Li et al. (Li et al. 2022) who reported that cinnamon supplementation reduced hepatic *SREBP-1c* expression, leading to decreased lipogenesis and improved hepatic steatosis. Similarly, Tuzcu et al. (Tuzcu et al. 2017) showed that cinnamon polyphenol extract inhibited *SREBP-1c* activation, which was associated with lower triglyceride levels and reduced inflammation in rats fed a HFD.

Furthermore, cinnamon appears to influence lipid and carbohydrate metabolism by modulating peroxisome proliferator-activated receptors (PPARs) (Desvergne and Wahli 1999, 2001). PPARs play an essential role in regulating lipogenic genes, with their activation promoting lipid accumulation through increased activity of lipoprotein lipase (Olefsky 2000). However, the effect of CNMA on PPARs appears to be context-dependent. A study by Huang et al. suggested that CNMA may downregulate the PPARs, thereby reducing adipogenesis and fat accumulation in HFD-fed mice and preventing further weight gain (Huang et al. 2011). In contrast, other studies, such as the study by Sheng et al., reported that cinnamon extracts can activate PPARs, leading to increased insulin sensitivity and decreased serum triglyceride levels (Sheng et al. 2008). These contrasting findings suggest that cinnamon metabolic effects may vary by compound and metabolic context. While PPAR activation can enhance insulin sensitivity and triglyceride clearance, its suppression may prevent lipid accumulation in advanced obesity.

Moreover, while cinnamon extract may activate PPARs, CNMA may selectively

inhibit *PPAR* expression in the liver and adipose tissue. This underscores the complexity of cinnamon metabolic effects and the need to distinguish between its active compounds.

Our findings also indicate that HFD feeding induced liver inflammation, as evidenced by elevated hepatic NOx levels. The CNMA administration effectively reduced NOx levels, returning them to the levels observed in the control rats. Increased NOx reflects inflammation and oxidative stress in metabolic diseases such as NAFLD (Gil-Cardoso et al. 2022). NOx modulates the NF-κB signaling, promoting expression of pro-inflammatory cytokines (Wu et al. 2024).

Inflammation plays a crucial role in the dysregulation of lipid metabolism, pathogenesis of obesity, and progression of NAFLD. Inflammatory signaling pathways, particularly those involving cytokines such as TNF-α (tumor necrosis factor-α) and IL-6 (interleukin-1), suppress PPAR expression, leading to insulin resistance and increased lipid accumulation. This process exacerbates obesity, metabolic disorders, and the development of NAFLD (Stienstra et al. 2007; Tai and Ding 2010; Masoodi et al. 2015; Wu et al. 2022). Research has shown that inflammatory cytokines can enhance HMG-CoA reductase activity, promoting cholesterol synthesis, a hallmark of several metabolic disorders (Weiss et al. 2020). This interaction between HMG-CoA reductase and inflammation may create a cyclical effect where elevated cholesterol further stimulates inflammation, thereby worsening conditions like NAFLD and obesity (Hu et al. 2022).

Our findings align with these observations, suggesting that CNMA can significantly reduce inflammatory marker concentrations (NOx and MDA), potentially contributing to its lipid-lowering effects (Zardast et al. 2023). Lee et al. demonstrated that CNMA inhibits the production of pro-inflammatory cytokines such as TNF-α and IL-1β, which are known to induce NO (nitric oxide) production (Lee et al. 2018). Similarly, a study by Gulec Peker and Kaltalioglu. found that CNMA treatment in lipopolysaccharide-activated macrophage cells significantly reduced NOx, TNF-α, IL-1β, and IL-6 production (Gulec Peker and Kaltalioglu 2021). Additionally, CNMA has been shown to suppress signaling pathways mediated by Toll-like receptor 4, a key regulator of inflammation often activated in obesity and metabolic syndrome (Liao et al. 2012; Lee et al. 2018). By modulating these inflammatory pathways, CNMA may help control inflammation which is closely linked to insulin resistance and NAFLD (Urasaki and Le 2022). Furthermore, our results indicate that CNMA treatment reduced NOx levels in the control animals receiving CNMA. This observation is consistent with evidence that certain herbal medicines and their bioactive constituents can exert both NO-mimetic and NO-lowering effects, particularly under non-pathological conditions (Braidy et al. 2010; Vattem and DeLeon 2013).

Our findings indicate that CNMA not only normalized serum liver enzyme levels and reduced hepatic triglyceride accumulation and liver weight (Yildiz et al. 2019; Xiao 2022) but also significantly improved liver histopathology. CNMA treatment preserved normal hepatic architecture and markedly decreased both fatty infiltration and steatosis, consistent with reports by Ustaoğlu et al. (Ustaoglu et al. 2023) and Luo et al. (Luo et al. 2021). These histopathological improvements reinforce our biochemical data, underscoring CNMA hepatoprotective efficacy, which is likely mediated by enhanced lipid metabolism and reduced oxidative stress.

In summary, our study demonstrates that CNMA provides significant hepatoprotection in a model of metabolic disturbance induced by a HFD. CNMA treatment markedly ameliorated key biochemical indicators of hepatic injury—including reductions in serum AST and

ALT, hepatic triglyceride accumulation, visceral fat mass, and markers of oxidative stress and inflammation—which were corroborated by histopathological analyses. Collectively, these results underscore the therapeutic potential of CNMA for mitigating NAFLD and associated metabolic disorders, providing a strong rationale for further mechanistic and clinical studies.

While this study provides valuable insights into the protective effects of CNMA against HFD-induced NAFLD, several limitations should be acknowledged. First, the relatively small sample size may reduce statistical power and limit the generalizability of the findings. Second, the study focused on short-term outcomes, and the long-term efficacy and safety of CNMA remain unexamined. Third, although key biochemical and histological markers were assessed, specific inflammatory cytokines such as NF-κB, TNF-α, and IL-6 were not measured. Additionally, gene expression analyses of regulatory targets implicated in lipid metabolism—such as SREBP-1c and HMG-CoA reductase—were not included. The absence of these molecular endpoints limits mechanistic interpretation. Future studies should incorporate a broader panel of inflammatory, oxidative stress, and gene expression markers to clarify CNMA mode of action and substantiate its therapeutic potential in metabolic disease.

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Conflicts of interest

The authors have no conflicts of interest.

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Ethical Considerations

The study involving animal subjects and ethics approval was conducted in accordance with the relevant guidelines at Birjand University of Medical Sciences.

Code of Ethics

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Authors' Contributions

KF designed the study and supervised the project at all steps. ZA and RF contributed to data collection. RF and KF contributed to professional manuscript writing and editing. MH performs the histopathological study. KF contributed to laboratory analysis and resources.

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