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Resveratrol attenuates hepatic oxidative stress and preserves gut mucosal integrity in high-fat diet-fed rats by modulating antioxidant and anti-inflammatory pathways

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This study evaluated the protective effects of resveratrol against high-fat diet (HFD)-induced hepatic dysfunction and intestinal mucus layer depletion with a focus on antioxidant and anti-inflammatory mechanisms. HFD-fed rats were treated with resveratrol for 8 weeks, and various metabolic, molecular, and histological parameters were assessed. Resveratrol therapy significantly reduced body weight gain and adiposity, lowered plasma and hepatic oxidative stress markers, and restored endogenous antioxidant enzyme activity. Liver function markers including ALT, AST, and ALP were normalized in treated animals. RT-PCR analysis showed enhanced expression of lipid-metabolizing and antioxidant genes including PPARα, CPT-1, Nrf2, and HO-1. Histological analysis revealed that resveratrol attenuated hepatic steatosis, collagen deposition, and fibrosis. Importantly, it preserved the goblet cell population within both intestinal crypts and villi, thereby maintaining the integrity of the gut-mucus barrier. These findings demonstrate that resveratrol exerts a multi-organ protective effect by simultaneously preserving intestinal mucus, improving hepatic antioxidant defenses, and reducing fibrosis. This study highlights a novel gut–liver axis mechanism for resveratrol action that extends beyond its well-known anti-obesity and mitochondrial benefits.

Keywords High fat diet, Oxidative stress, Inflammation, Resveratrol, Non-alcoholic fatty liver disease, Intestinal mucosal barrier

The consumption of high-fat, calorie-dense foods typical of the Western diet has been strongly associated with the rise in obesity and metabolic disorders globally[1,2]. Among the most concerning consequences of the high-fat diet (HFD) is its role in inducing hepatic oxidative stress, a key factor in the development of non-alcoholic fatty liver disease (NAFLD)[3,4]. NAFLD is the most common chronic liver condition, characterized by fat accumulation, oxidative damage, and inflammation in the liver, which can progress to more severe liver diseases, such as steatohepatitis and fibrosis[5]. Importantly, diet-induced hepatic dysfunction often leads to systemic inflammation, exacerbating metabolic syndrome, insulin resistance, type 2 diabetes, and cardiovascular diseases[6–8].

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Despite the alarming increase in the prevalence of NAFLD and its associated metabolic dysfunctions, effective treatments remain limited. Current therapeutic strategies are largely focused on lifestyle modifications and symptomatic management rather than targeting the specific underlying pathophysiological mechanisms of oxidative stress and inflammation[9,10]. This has prompted growing interest in the use of natural products with antioxidant and anti-inflammatory properties to counteract hepatic oxidative stress and inflammation leading to NAFLD and other harmful metabolic disorders arising due to the consumption of HFD[11–13].

Resveratrol, a polyphenol abundant in various plants such as grapes, berries, and peanuts, has garnered significant attention for its potential therapeutic benefits in the context of metabolic and inflammatory disorders, including those caused by HFD[14]. This therapeutic benefit is primarily attributed to its antioxidant and anti-inflammatory properties[15,16]. Given NAFLD and chronic metabolic conditions are linked to inflammation and oxidative stress, resveratrol is promising due to its ability to enhance the body's antioxidant defenses while suppressing key pro-inflammatory signaling pathways, including those mediated by NF-kB. Extensive research using in vitro, ex vivo, and animal models has demonstrated the beneficial actions of resveratrol in cancer chemoprevention and therapy[17–19] cardiovascular diseases and obesity[20–25] metabolic dysregulation (both alcoholic and non-alcoholic)[26–31] diabetes[21,30] arthritis[32] wound healing[33] osteoporosis, and neuroprotection[34,35].

While resveratrol's anti-obesity and mitochondrial benefits are well-documented, its effects on the gut–liver axis remain underexplored. Specifically, little is known about its ability to protect the intestinal epithelial barrier, which plays a critical role in preventing systemic inflammation and hepatic injury. Disruption of this barrier, particularly through the loss of mucus-producing goblet cells, has been increasingly linked to enhanced intestinal permeability, endotoxemia, and progression of liver disease[36]. Despite this, previous studies have not addressed whether resveratrol can preserve intestinal goblet cell populations or protect the mucus layer under conditions of long-term HFD stress.

To address this gap, the present study evaluated the effects of resveratrol on HFD-induced hepatic oxidative stress and systemic inflammation, with a particular focus on its potential to preserve intestinal barrier integrity. The findings demonstrated that resveratrol not only prevented HFD-induced weight gain and fat accumulation but also significantly reduced oxidative stress markers in both plasma and liver tissues. Furthermore, resveratrol preserved the activity of endogenous antioxidant enzymes and prevented the elevation of liver function markers, underscoring its role in protecting liver health. Importantly, the study revealed that resveratrol enhanced the expression of fat-metabolizing and antioxidant genes, attenuated hepatic steatosis, collagen deposition, and fibrosis, and reversed the decline in mucus-producing goblet cells in the intestine, which are critical for maintaining barrier function and preventing systemic inflammation.

Overall, this dual role of resveratrol, mitigating liver oxidative stress and preserving intestinal barrier function, presents a novel therapeutic mechanism that targets the gut-liver axis. These findings extend the scope of resveratrol's benefits beyond its established mitochondrial effects and support its relevance as a multi-targeted agent in preventing metabolic and inflammatory consequences of a high-fat Western diet, including NAFLD and associated comorbidities.

Materials and methods
Chemicals
Beef tallow, used in the preparation of the high-fat diet (HFD), was sourced from Dhaka New Market, Bangladesh. Reagent kits for the measurement of alanine aminotransferase (ALT), alkaline phosphatase (ALP), triglycerides, total cholesterol, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) were obtained from DCI Diagnostics (Budapest, Hungary). Thiobarbituric acid (TBA) was acquired from Sigma-Aldrich (St. Louis, MO, USA), while resveratrol was purchased from Xi'an Surnature Biological Technology Co. Ltd, China.

Animals and treatment protocols
All animal experiments were conducted in compliance with the guidelines of the National Institutes of Health (NIH, USA) and The American Veterinary Medical Association (AVMA) and were approved by the Institutional Animal Care and Use Committee (IACUC) at North South University, Dhaka, Bangladesh (Approval No. AEC 006-2017) and all experiments were reported in compliance with the ARRIVE guidelines. The study utilized twenty-eight male Wistar rats, aged 10–12 weeks and weighing between 175 and 210 g, sourced from the Animal House of the Department of Pharmaceutical Sciences, North South University, Dhaka. The animals were housed individually under controlled conditions, maintained at a temperature of 22 ± 2 °C, 55% humidity, and a 12-h light/dark cycle.

The rats were randomly assigned to four experimental groups, each comprising seven animals. The composition of the standard diet and high-fat diet (HFD) (Suppl. Table 1) used in this experiment is detailed in a previous publication[37]. Group I (Control) received a standard diet containing 14 kcal% proteins, 57 kcal% carbohydrates, and 13.5 kcal% fats. Group II (Control + RSV) was given the same standard diet al.ong with resveratrol at a dose of 100 mg/kg daily for 8 weeks. A dose of 100 mg/kg daily was chosen as it corresponds to a dose of 1 g/day for humans which has been shown to be therapeutically beneficial and well-tolerated[38,39]. Group III (HF) was fed an HFD consisting of 14 kcal% proteins, 37 kcal% carbohydrates, and 48 kcal% fats. Group IV (HF + RSV) received the HFD along with resveratrol at 100 mg/kg daily for the same duration.

Resveratrol, mixed with olive oil, was administered to rats in Groups II and IV via oral gavage, while Groups I and III received olive oil alone as a vehicle control, also via oral gavage. All animals had unrestricted access to food and water throughout the study. An oral glucose tolerance test (OGTT) was performed on all groups prior to the beginning of the study and repeated at the end of the 8-week treatment period to assess the effects of HFD on insulin resistance. Additionally, daily measurements of body weight, food intake, and water consumption were recorded for each rat.

Euthanasia and tissue harvesting

After 8 weeks, all animals were euthanized with an intraperitoneal injection of pentobarbital (65 mg/kg). Blood samples were collected in heparinized tubes, and within 30 min of collection, the samples were centrifuged at 7000 g for 15 min at 4 °C to separate the plasma. The isolated plasma was then stored at −20 °C for subsequent analysis. The liver, intestine, and various adipose tissues, including mesenteric, peritoneal, and epididymal fat, were surgically excised and weighed. For histological examination, the organs were immediately preserved in neutral buffered formalin (PH 7.4). All other tissue samples were stored at −20 °C for further biochemical studies. Liver tissues were homogenized to facilitate biochemical analyses.

Oral glucose tolerance test (OGTT)

The oral glucose tolerance test (OGTT) was performed both before and after the initiation of HFD feeding, following previously described protocols[37]. In brief, rats were fasted for approximately 12 h overnight, with access to water maintained during the fasting period. Baseline blood glucose levels were measured from tail vein samples using a glucometer (Bionim Corporation, Bedford, MA, USA). Each rat then received an oral gavage of glucose at a dose of 2 g/kg, prepared as a 40% aqueous solution. Blood glucose levels were subsequently measured from the tail vein at 30, 60, 90, and 120 min after glucose administration, using the same glucometer (Bionim Corporation, Bedford, MA, USA).

Assessment of liver function markers

The activities of liver function marker enzymes, including alanine aminotransferase (ALT) and alkaline phosphatase (ALP), were measured in plasma using diagnostic kits from DCI Diagnostics (Budapest, Hungary), following the instructions provided by the manufacturer.

Measurement of plasma cholesterol and triglyceride levels

Plasma concentrations of cholesterol and triglycerides were determined using assay kits from DCI Diagnostics (Budapest, Hungary), following the instructions provided by the manufacturer.

Determination of oxidative stress markers

Liver tissues were homogenized in phosphate buffer solution (PBS) at Ph 7.4, and the homogenate was centrifuged at 7000 g for 15 min at 4 °C. The supernatant was collected to assess oxidative stress markers in both tissue and plasma samples.

Estimation of lipid peroxidation

Lipid peroxidation, an indicator of oxidative stress, was assessed by measuring thiobarbituric acid reactive substances (TBARS) in plasma and liver tissues, following a previously described method[40]. Briefly, 0.1 Ml of liver tissue homogenate or plasma was mixed with 2 Ml of a reagent solution containing thiobarbituric acid (TBA), acetic acid, and HCl (1:1:1) and incubated in a hot water bath for 15 min. After cooling to room temperature, the absorbance of the clear supernatant was measured at 535 nm using a 96-well plate reader (Thermo Scientific, Original Multiskan EX, USA).

Assay of nitric oxide (NO)

Nitric oxide levels in plasma and liver tissue homogenates were quantified using modified Griess-Illosvoy reagents, as described in previous studies[41,42]. Plasma or tissue homogenates were added to the reaction mixture and incubated for 150 min at 25 °C, resulting in the development of a pink chromophore. The absorbance was measured at 540 nm on a 96-well plate reader (Thermo Scientific, Original Multiskan EX, USA) against a blank solution. NO levels were reported in nmol/Ml or nmol/g of tissue, calculated based on a standard curve.

Advanced protein oxidation products (APOP) assay

The APOP levels were determined following methods described in earlier publications[43,44]. Plasma samples were diluted 5:1 with PBS, and 0.1 Ml of 1.16 M potassium iodide (KI) was added to each tube. After 2 min, 0.2 Ml of acetic acid was added. The absorbance of the mixture was immediately recorded at 340 nm after a blank containing PBS, KI, and acetic acid was prepared. Measurements were performed on a 96-well plate reader (Thermo Scientific, Original Multiskan EX, USA). APOP concentrations were expressed as μmol L^{-1} chloramine-T equivalents, with absorbance being linear from 0 to 100 mmol/L.

Catalase (CAT) activity assay

Catalase activity in plasma and liver tissue homogenates was measured based on protocols from earlier studies[42,45]. The reaction mixture included 50 mmol phosphate buffer (Ph 5.0), 5.9 mmol hydrogen peroxide, and 0.1 Ml of the sample (plasma or tissue homogenate). The change in absorbance at 240 nm was monitored, with a change of 0.01 units/min defined as one unit of CAT activity. Readings were taken using a 96-well plate reader (Thermo Scientific, Original Multiskan EX, USA).

Estimation of superoxide dismutase (SOD) activity

The SOD activity was assessed in plasma and liver homogenates using a method previously reported[42,46]. The reaction mixture was prepared, and absorbance was monitored at 480 nm for 1 min at 15-s intervals on a 96-well plate reader (Thermo Scientific, Original Multiskan EX, USA). A blank containing no tissue homogenate was run alongside the samples. SOD activity was defined as the amount of enzyme required to inhibit the auto-oxidation of epinephrine by 50%0.2.10 Gene Expression Analysis by Quantitative Real-Time PCR Thermal Cycler (Bio-Rad, USA). The expression levels of oxidative stress and inflammation-related genes were measured

by quantitative real-time PCR (Qpcr) using Maxima SYBR Green Qpcr Master Mix (Thermo Fisher Scientific, USA) on a CFX96 C1000 Touch Real-Time PCR Detection System (Bio-Rad, USA). Data analysis was conducted using CFX Manager Software, following the manufacturer's protocols. Qpcr was performed based on the procedure outlined by Khan et al.[47] with forward and reverse primers designed using Primer 3 online software (Suppl. Table 2). Gene expression levels were normalized to the expression of β-actin within the same sample.

Histopathological examination

Liver tissues were fixed in neutral buffered formalin, embedded in paraffin, and sectioned at a thickness of 5 µm using a rotary microtome. Sections were stained with hematoxylin and eosin (H&E) to assess structural changes and inflammatory cell infiltration in hepatic tissue, and steatosis grading was performed for each group. Additionally, liver sections were stained with Picrosirius red to evaluate the presence of fibrosis, and the extent of fibrosis was quantified using ImageJ software (NIH, USA). Prussian blue staining was conducted to examine iron deposition within the tissues. Images of the stained sections were captured at 10x or 40x magnification using a Zeiss Axioscope light microscope, and processed for further analysis.

Statistical analysis

All statistical analyses were performed using GraphPad Prism software (version 6.0) and figures made using OriginLab software (version 2019b)[48,49]. Data are expressed as mean ± standard error of the mean (SEM). Comparisons between groups were conducted using one-way ANOVA followed by Tukey's post hoc test. Specific comparisons were made between the Control and HF groups, as well as between the HF and HF + RSV group. A p-value of < 0.05 was considered to indicate statistical significance in all analyses.

Results

Effects of Resveratrol on body weight, food, and water intake

The daily food and water intake along with body weight changes for the rats in the various experimental groups are presented in Table 1. Across all experimental groups, daily food and water intake remained comparable. However, rats fed a high-fat diet (HFD) consumed significantly more calories, resulting in a substantial increase in body weight compared to those on a control diet. Importantly, resveratrol treatment mitigated the weight gain in HFD-fed rats.

Resveratrol improves impaired glucose metabolism in HFD-fed rats

The results of the oral glucose tolerance test (OGTT), used to assess glucose metabolism, are shown in Fig. 1. At the beginning of the study, all four experimental groups displayed similar basal glucose concentrations, approximately 4 mmol/L (Fig. 1A). Following glucose administration, glucose levels peaked at 30 min across all groups and gradually returned to basal levels by 120 min indicating normal glucose utilization and clearance from the blood. After 56 days, all groups except for the HFD group again exhibited the similar basal glucose levels, which rose 30 min after glucose administration. In the HFD-fed group, glucose levels remained elevated and did not return to baseline within 120 min, unlike the control group, which normalized within 60–120 min. Notably, resveratrol treatment significantly reduced blood glucose levels in the HFD + RSV group compared to the HFD group (Fig. 1B), demonstrating a marked improvement in glucose utilization. The area under the curve (AUC) analysis at 120 min revealed a significantly higher glucose excursion in the HFD group, whereas resveratrol-treated and control groups exhibited comparable AUC values, indicating improved glucose tolerance relative to the HFD group (Fig. 1C). These findings demonstrate that resveratrol improves impaired glucose metabolism in HFD-fed rats.

Resveratrol administration reduces fat deposition in HFD-fed rats

As expected, the data show that HFD feeding led to a marked increase in fat accumulation in the peritoneal, mesenteric, and epididymal regions compared to controls (Fig. 2). Resveratrol treatment significantly reduced fat deposition in the peritoneal and mesenteric regions of HFD-fed rats, bringing these levels closer to those seen in the control group. However, resveratrol did not significantly affect epididymal fat accumulation in HFD-fed rats, which remained elevated compared to controls (Fig. 2).

	Control	Control + RSV	HFD	HFD + RSV
Initial body weight (g)	188.49 ± 4.17	187.03 ± 1.66	202.37 ± 1.23	200.09 ± 2.65
Final body weight (g)	220.97 ± 4.79	220.93 ± 4.09	329.86 ± 5.70*	279.67 ± 12.78*#
Food intake (g/day)	14.55 ± 1.30	15.89 ± 1.18	12.28 ± 1.21	14.70 ± 1.06
Water intake (mL/day)	17.61 ± 1.92	16.12 ± 1.28	14.14 ± 1.38	13.89 ± 0.90

Table 1. Effect of Resveratrol on body weight, food and water intake and calorie intake in HFD-fed rats. Data are presented as Mean ± SEM, $n = 7$. Statistical analysis was performed by One Way ANOVA with Tukey's post hoc test. * $p < 0.05$ vs. Control, # $p < 0.05$ vs. HFD.
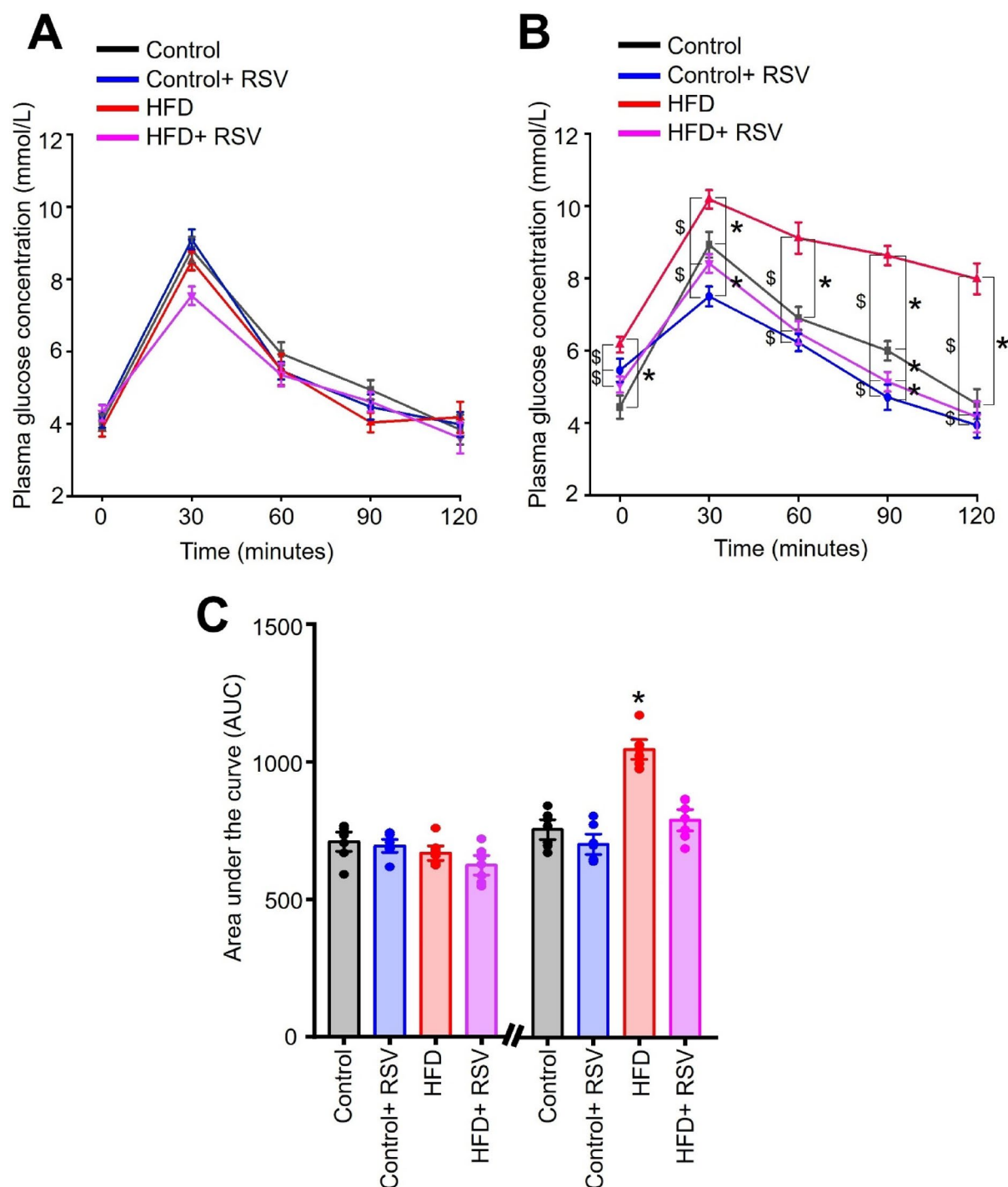
Fig. 1. Effect of resveratrol on glucose intolerance in HFD-fed rats. (**A**) Mean data from the oral glucose tolerance test (OGTT) before placing the animals on a high-fat diet. (**B**) Mean data from the OGTT after 56 days of HFD feeding. (**C**) Area under the curve (AUC) for all the groups calculated at 120 min. Data are presented as mean ± SEM, with $N = 7$. Statistical comparisons were made using one-way ANOVA followed by Tukey's post-hoc test. * $p < 0.05$ vs. Control, \$ $p < 0.05$ vs. HFD.

Resveratrol supplementation inhibits the elevation of plasma ALT, AST, ALP, and MPO enzyme activities in HFD rats

Elevated plasma levels of ALT, AST, and ALP are critical markers of liver dysfunction and hepatocellular damage. The data demonstrate that HFD-fed rats exhibited significantly elevated levels of these enzymes compared to the control group, indicating hepatic impairment (Fig. 3A–C). However, resveratrol supplementation for 8 weeks significantly reduced the activity of ALT, AST, and ALP in HFD-fed rats ($p < 0.05$), suggesting a protective
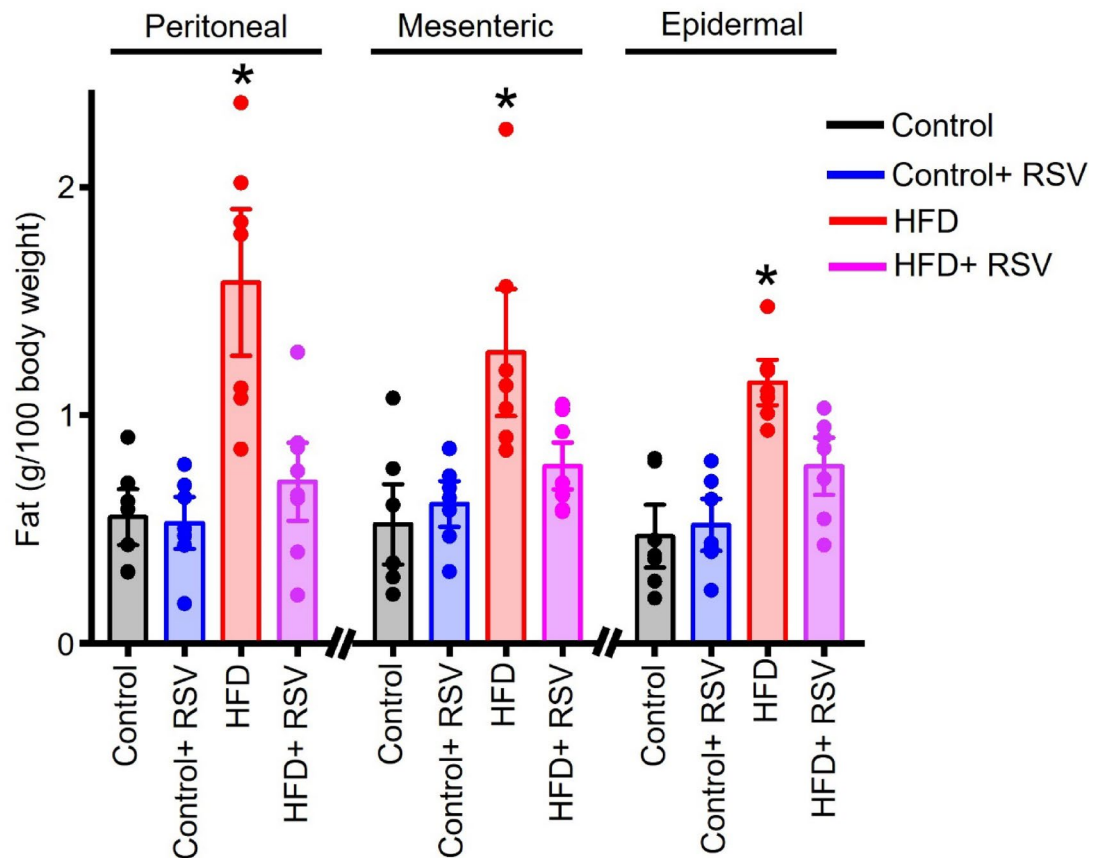
Fig. 2. Effect of resveratrol on fat deposition in HFD-fed rats. Data are presented as mean ± SEM, with $N = 7$. Statistical comparisons were made using one-way ANOVA followed by Tukey's post-hoc test. * $p < 0.05$ vs. Control.

effect against HFD-induced liver damage. Notably, resveratrol had no effect on enzyme levels in control rats, indicating its specific role in mitigating HFD-related liver dysfunction (Fig. 3). In addition, myeloperoxidase (MPO) activity was measured in the liver, as plasma MPO measurement can be confounded by several factors. Liver MPO activity was significantly elevated in HFD-fed rats, but resveratrol treatment restored MPO levels to those observed in the control group (Fig. 3D). These findings further support the hepatoprotective effects of resveratrol in HFD-induced liver dysfunction.

Resveratrol treatment suppresses the elevation of total cholesterol levels in HFD-rats

To assess whether resveratrol could counteract the HFD-induced rise in total cholesterol, plasma cholesterol levels were measured in all groups. HFD-fed rats exhibited a sharp increase in total plasma cholesterol compared to controls (Fig. 3E). However, this rise was significantly blunted in HFD-fed rats treated with resveratrol for 8 weeks, indicating that resveratrol can mitigate the hypercholesterolemic effects of a HFD.

Resveratrol treatment attenuates HFD-induced oxidative stress

The data demonstrate that HFD is associated with increased oxidative and nitrosative stress markers, including malondialdehyde (MDA), advanced protein oxidation products (APOP), and elevated nitric oxide (NO) levels in the liver (Fig. 4). These findings suggest that HFD-induced hepatocellular damage leads to elevated concentrations of these stress markers in both liver tissue and plasma. Importantly, resveratrol supplementation significantly attenuated the HFD-associated increases in MDA, NO, and APOP levels, as observed in both liver tissue homogenates and plasma samples (Fig. 4). Collectively, these results indicate that resveratrol has potent in vivo antioxidant properties, scavenges free radicals and reactive oxygen and nitrogen species, and thereby provides protective effects against oxidative damage in the liver.

Resveratrol preserves endogenous antioxidant activities in HFD-rats

Naturally occurring endogenous antioxidants, such as superoxide dismutase (SOD), catalase, and reduced glutathione (GSH), act as defenses against oxidative damage. However, during conditions of oxidative stress, the levels of these antioxidants are often depleted. The findings reveal that rats fed a HFD exhibited significantly reduced SOD and catalase activity (Fig. 5), indicating compromised antioxidant defenses, a sign of oxidative stress. Resveratrol treatment potently prevented this decline, maintaining antioxidant enzyme activity at levels comparable to controls. Similarly, GSH, a key non-enzymatic antioxidant, was depleted in HFD-fed rats, but
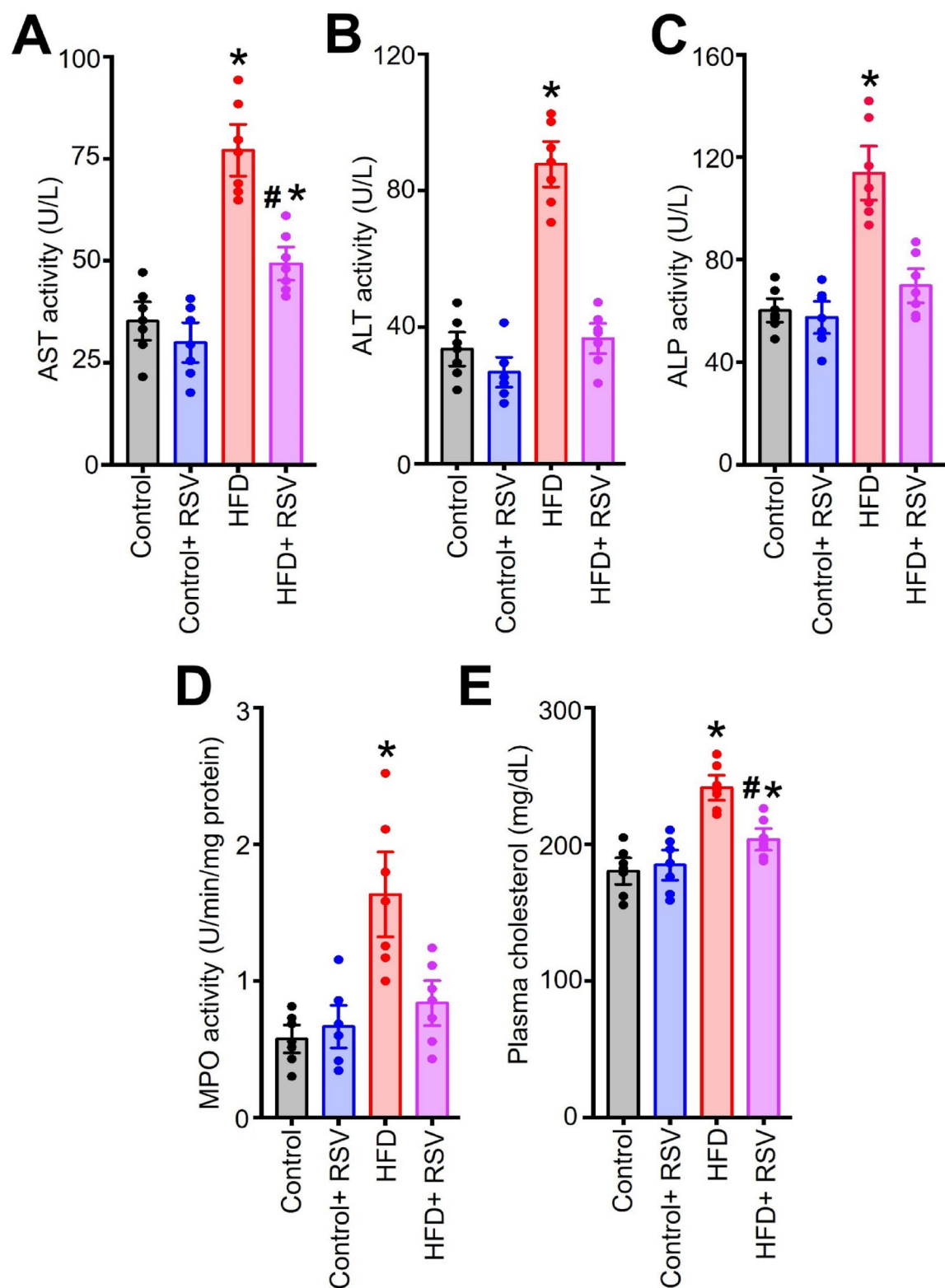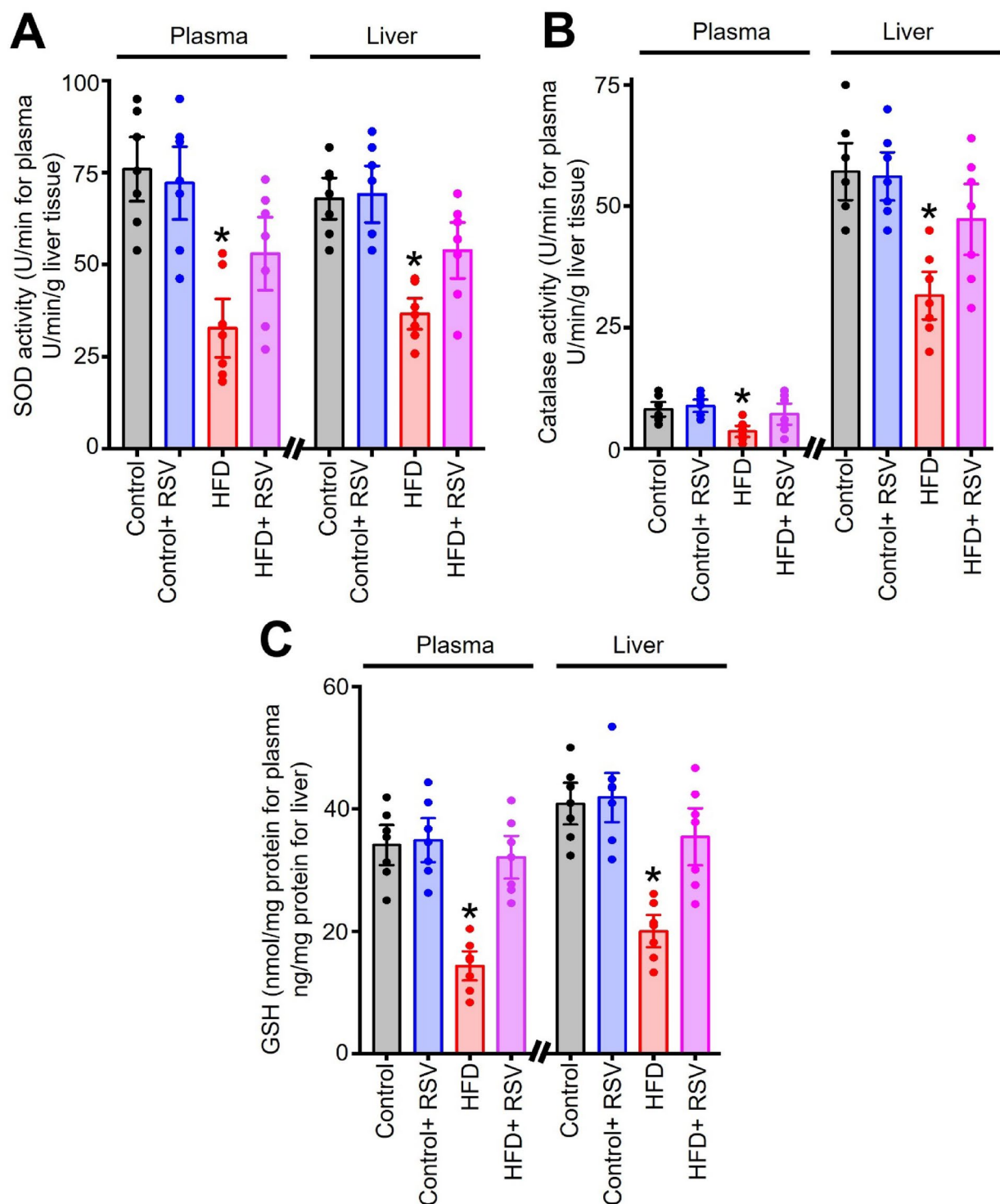
Fig. 3. Effect of resveratrol on plasma enzyme activities and cholesterol levels in HFD-fed rats. Data are presented as mean ± SEM, with $N = 7$. Statistical comparisons were made using one-way ANOVA followed by Tukey's post-hoc test. * $p < 0.05$ vs. Control, # $p < 0.05$ vs. HFD.
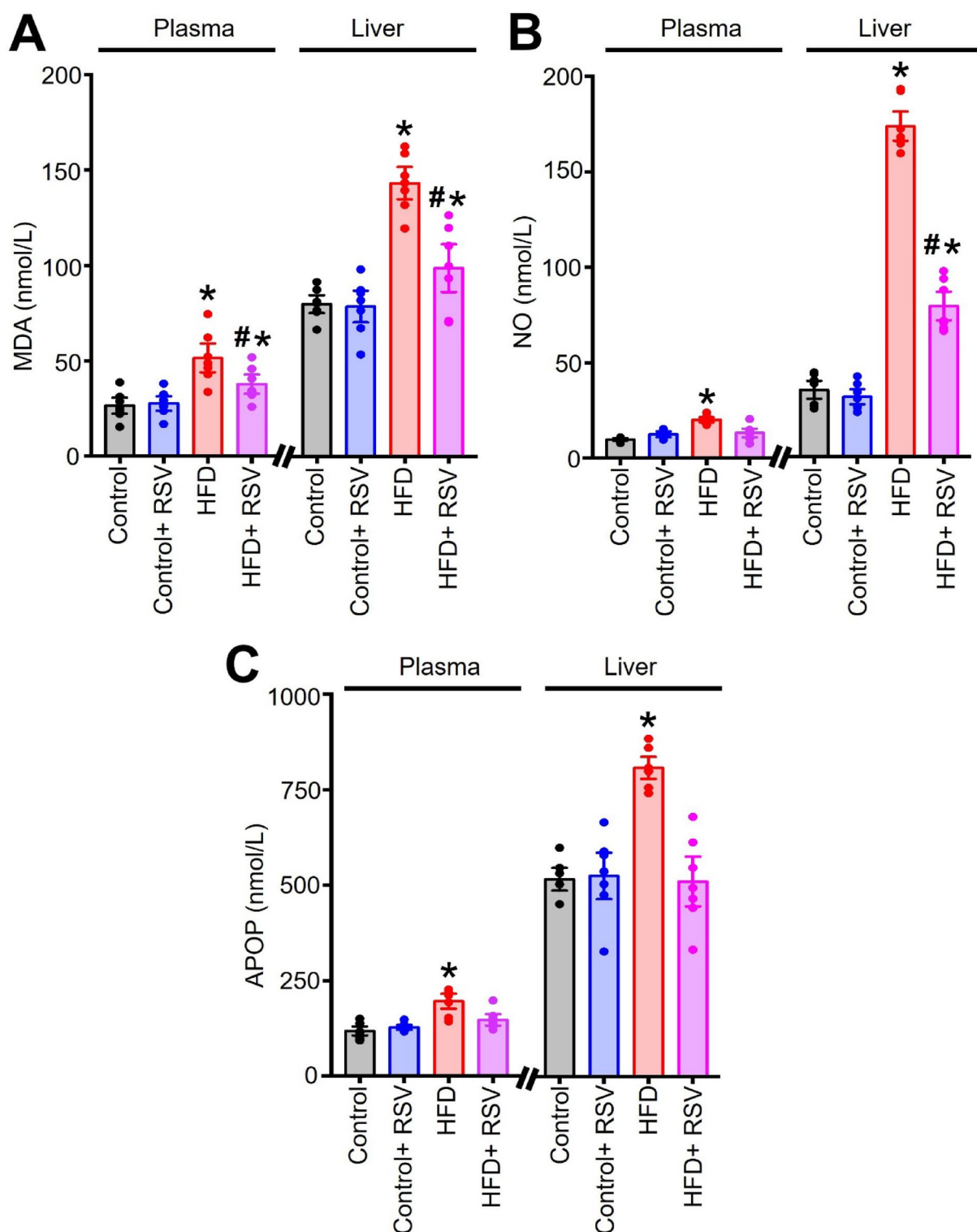
Fig. 4. Effect of resveratrol on oxidative stress markers in plasma and liver of HFD-fed rats. Data are presented as mean ± SEM, with $N = 7$. Statistical comparisons were made using one-way ANOVA followed by Tukey's post-hoc test. * $p < 0.05$ vs. Control, # $p < 0.05$ vs. HFD.

Fig. 5. Effect of resveratrol treatment on antioxidant enzyme activities in plasma and liver of HFD-fed rats. Data are presented as mean ± SEM, with $N = 7$. Statistical comparisons were made using one-way ANOVA followed by Tukey's post-hoc test. * $p < 0.05$ vs. Control, # $p < 0.05$ vs. HFD.

resveratrol co-treatment maintained GSH levels to those observed in control animals (Fig. 5). These data suggest that resveratrol acts as a dual-action antioxidant, both preserving endogenous antioxidant systems and mitigating oxidative and nitrosative stress.

Resveratrol upregulates antioxidant gene expressions in the liver

The powerful antioxidant activity of resveratrol against HFD-induced oxidative stress prompted an investigation into whether resveratrol influenced the expression of antioxidant genes in the liver of HFD-fed rats. The data

revealed a substantial decrease in the Mrna levels of Nrf-2 and its associated genes, including HO-1, HO-2, and GPx, in the liver of HFD-fed rats compared to controls (Fig. 6). Resveratrol treatment remarkably prevented this downregulation, maintaining the expression of these genes at levels similar to those in control rats. Consistent with these results, the mRNA expression of other key antioxidant enzymes, such as catalase and SOD, was also significantly reduced in the liver of HFD-fed rats. However, resveratrol administration restored the expression of catalase and SOD to near-normal levels (Fig. 6). These findings suggest that resveratrol not only acts as a direct antioxidant but also promotes the upregulation of endogenous antioxidant defense mechanisms by modulating the expression of critical antioxidant genes.

Resveratrol modulates the gene expression of adipogenic transcription factors and metabolizing enzymes

To further explore the metabolic effects of resveratrol, the study examined the expression of key genes involved in lipid metabolism and fat deposition in the liver of HFD-fed rats. The results showed a significant elevation in the Mrna levels of peroxisome proliferator-activated receptor gamma (PPARγ), CCAAT-enhancer-binding protein alpha (C/EBPα), sterol regulatory element-binding protein (SREBP)1c, and leptin – all of which promote fat storage in tissues, including the liver (Fig. 7). Resveratrol treatment reduced the expression of PPARγ, C/EBPα, and SREBP1c, indicating a decrease in lipid accumulation (Fig. 7). However, resveratrol did not alter leptin expression in the HFD + RSV group compared to HFD-fed rats (Fig. 7). Conversely, a significant decrease in the Mrna levels of AMPK, adiponectin, AdipoR1, and GLUT-4, which are important for lipid metabolism, was observed in the liver of HFD-fed rats (Fig. 7). Resveratrol administration upregulated the expression of AMPK and adiponectin, suggesting an improvement in metabolic regulation (Fig. 7). However, the HFD-induced downregulation of AdipoR1 and GLUT-4 expression was not reversed by resveratrol treatment (Fig. 7). These findings highlight the critical role of resveratrol in modulating lipid metabolism by reducing fat deposition and enhancing certain metabolic pathways, although its effects on specific metabolic regulators like AdipoR1 and GLUT-4 remain limited.

Resveratrol downregulates Proinflammatory gene expressions in the liver

To investigate the anti-inflammatory effects of resveratrol in the context of HFD-induced liver inflammation, the expression of key inflammatory markers in HFD-fed and control rats was analyzed. HFD-fed rats showed a significant increase in liver inflammation compared to those on a control diet, marked by elevated levels of the pro-inflammatory cytokines IL-1 and IL-6 ($p < 0.05$) (Fig. 8A). Resveratrol administration suppressed this upregulation, reducing the expression of IL-1 and IL-6 in HFD-fed rats to levels closer to those in control animals. Importantly, resveratrol treatment in control diet-fed rats did not alter IL-1 and IL-6 gene expression, suggesting its anti-inflammatory effects are specific to the inflammatory state induced by HFD (Fig. 8A). Additionally, HFD led to a significant upregulation of TNF-α Mrna expression compared to control rats ($p < 0.05$), a change that was prevented by resveratrol treatment in HFD-fed rats (Fig. 8B). The data also showed significantly higher expression of TGF-β Mrna in the liver of HFD-fed rats ($p < 0.05$) compared to controls, indicative of increased fibrosis and inflammatory response (Fig. 8B). The expression of Inos, a key enzyme linked to inflammation and nitrosative stress, was also markedly upregulated ($p < 0.05$) in the liver of HFD-fed rats compared to control rats (Fig. 8B). Resveratrol treatment inhibited this rise in Inos expression, suggesting its role in mitigating nitrosative stress in HFD-fed rats (Fig. 8B).

In line with these findings, NF-κB mRNA expression, a critical regulator of inflammatory cytokines, was significantly elevated in the liver of HFD-fed rats compared to control rats (Fig. 8C). Resveratrol administration reduced NF-κB expression in HFD-fed rats, preventing the activation of inflammatory signaling pathways associated with HFD-induced liver inflammation (Fig. 8C). Overall, these results demonstrate that resveratrol has potent anti-inflammatory effects, reducing the expression of key inflammatory markers and signaling pathways activated by HFD, thereby offering a protective effect against liver inflammation.

Resveratrol ameliorates HFD-induced histopathological changes in the liver

To further assess the protective effects of resveratrol against HFD-induced tissue damage, histological analyses of liver and intestine sections from control and HFD-fed rats were performed. Photomicrographs of liver sections from control rats showed typical tissue architecture, with no abnormal fat deposits, necrosis, or infiltration of inflammatory cells (Fig. 9). In contrast, liver sections from HFD-fed rats exhibited clear signs of hepatic degeneration, characterized by the accumulation of excessive fat droplets and infiltration of inflammatory cells.

Remarkably, resveratrol treatment effectively suppressed these HFD-induced changes, preserving normal liver structure (Fig. 9A–E). The scoring of hepatic steatosis is provided in Fig. 9F. Since oxidative stress induced by HFD can lead to liver fibrosis, Picrosirius red staining was conducted on liver sections to evaluate collagen deposition. Control rats displayed normal collagen distribution and alignment, indicating an absence of fibrosis (Fig. 10). In HFD-fed rats, there was a significant accumulation of collagen, consistent with the development of fibrosis. However, this was potently inhibited by resveratrol treatment, as shown by reduced collagen staining in the liver (Fig. 10). Figure 10E shows the percentage of hepatic fibrosis associated with HFD feeding with or without resveratrol treatment. These findings suggest that resveratrol possesses strong anti-fibrotic effects, likely due to its in vivo antioxidant and anti-inflammatory properties. Periodic acid-Schiff (PAS) staining was also performed on intestinal sections to examine intestinal histology (Fig. 11).

Resveratrol protects intestinal goblet cell populations from HFD-induced depletion

Photomicrographs from control rats revealed a normal distribution of mucus-producing goblet cells within the intestinal villi and crypts, essential for maintaining the protective mucus layer that shields the epithelium from mechanical damage, pathogens, and inflammation. In contrast, HFD-fed rats exhibited a marked reduction in
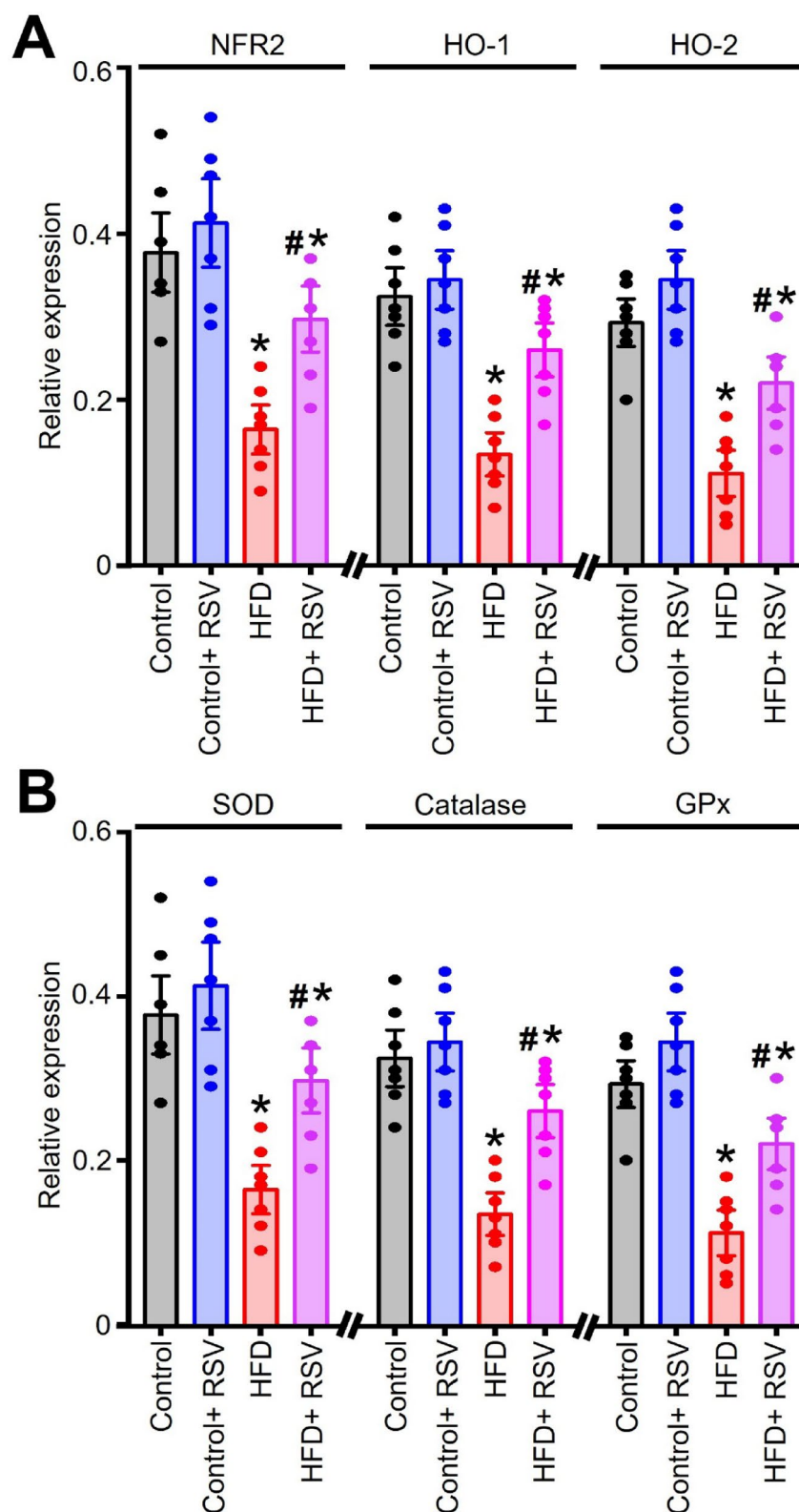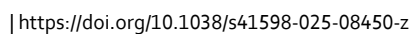
Fig. 6. Effect of resveratrol on oxidative stress-related genes in the liver of HFD-fed rats compared to GAPDH. Data are presented as mean ± SEM, with $N = 6$. Statistical comparisons were made using one-way ANOVA followed by Tukey's post-hoc test. * $p < 0.05$ vs. Control, # $p < 0.05$ vs. HFD.
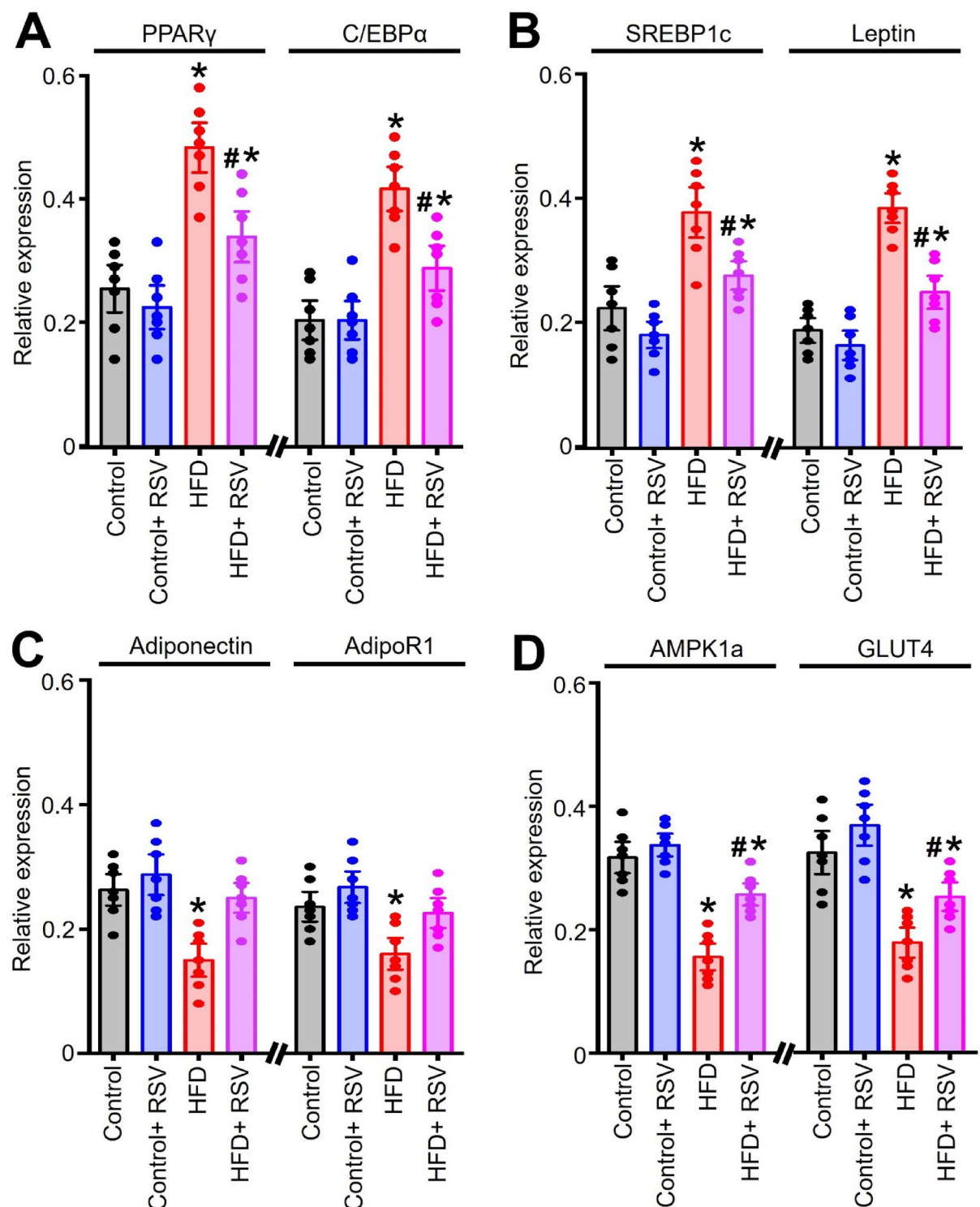
Fig. 7. Effect of resveratrol on fat metabolizing genes in liver of HFD-fed rats. $N = 6$. Data are presented as mean ± SEM. Statistical comparison was made by One Way ANOVA followed by Tukey's post-hoc test. * $p < 0.05$ vs. Control, # $p < 0.05$ vs. HFD.

goblet cell populations, indicative of compromised intestinal barrier integrity. Resveratrol treatment preserved goblet cell density in HFD-fed rats, effectively maintaining the structural and functional integrity of the intestinal mucus layer (Fig. 11). These findings suggest that resveratrol protects against HFD-induced depletion of goblet cells and prevents associated damage to the intestinal epithelium. In parallel, resveratrol also inhibited oxidative injury and fibrosis in the liver. Together, these results highlight the ability of resveratrol to preserve gut–liver axis integrity by mitigating oxidative stress, inflammation, and epithelial degeneration in both organs.

Fig. 8. Effect of resveratrol on inflammatory gene expression in the liver of HFD-fed rats. Data are presented as mean ± SEM, with *N* = 6. Statistical comparisons were made using one-way ANOVA followed by Tukey's post-hoc test. * *p* < 0.05 vs. Control, # *p* < 0.05 vs. HFD.
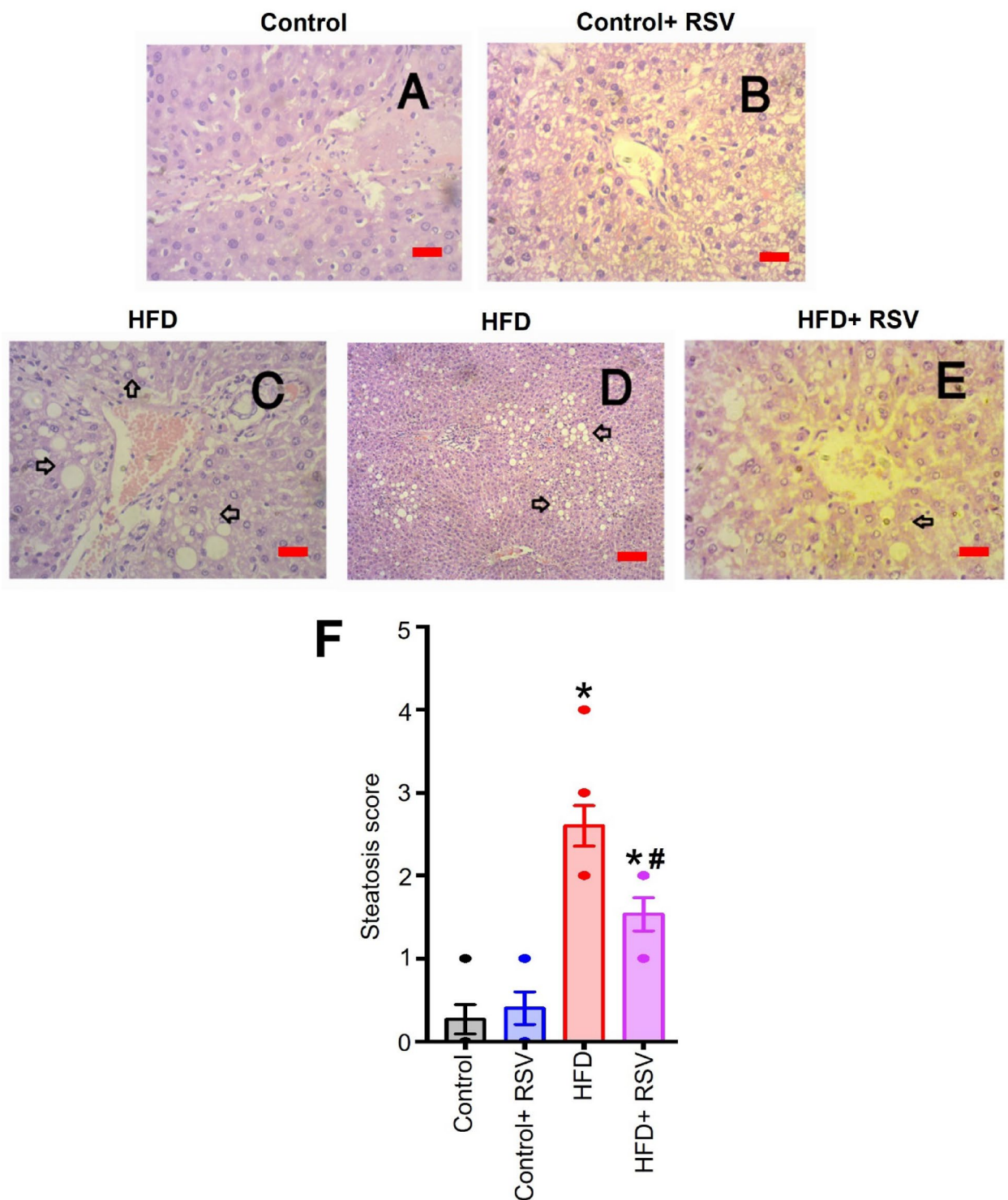
Fig. 9. Effect of resveratrol on fat droplet deposition in the liver of HFD-fed rats. (**A**) Control group: no signs of inflammation, necrosis, or fat droplet deposition in liver sections. (**B**) Resveratrol-treated control group: no inflammation, necrosis, or fat droplet deposition observed. (**C**) High-fat diet (HFD) group (40X magnification): liver sections showed evidence of inflammation, necrosis, and fat droplet deposition. (**D**) High-fat diet (HFD) group (10X magnification): liver sections showed evidence of fat droplet deposition. I HFD with resveratrol treatment: no signs of inflammation, necrosis, or fat droplet deposition in liver sections. Images were captured at 40X magnification. Scale bar in (**A**–**E**) 100 μm. (**F**) Steatosis scores demonstrating resveratrol treatment significantly reduced steatosis in HFD-fed rats. Statistical comparisons were made using one-way ANOVA followed by Tukey's post-hoc test. * $p < 0.05$ vs. Control, # $p < 0.05$ vs. HFD.
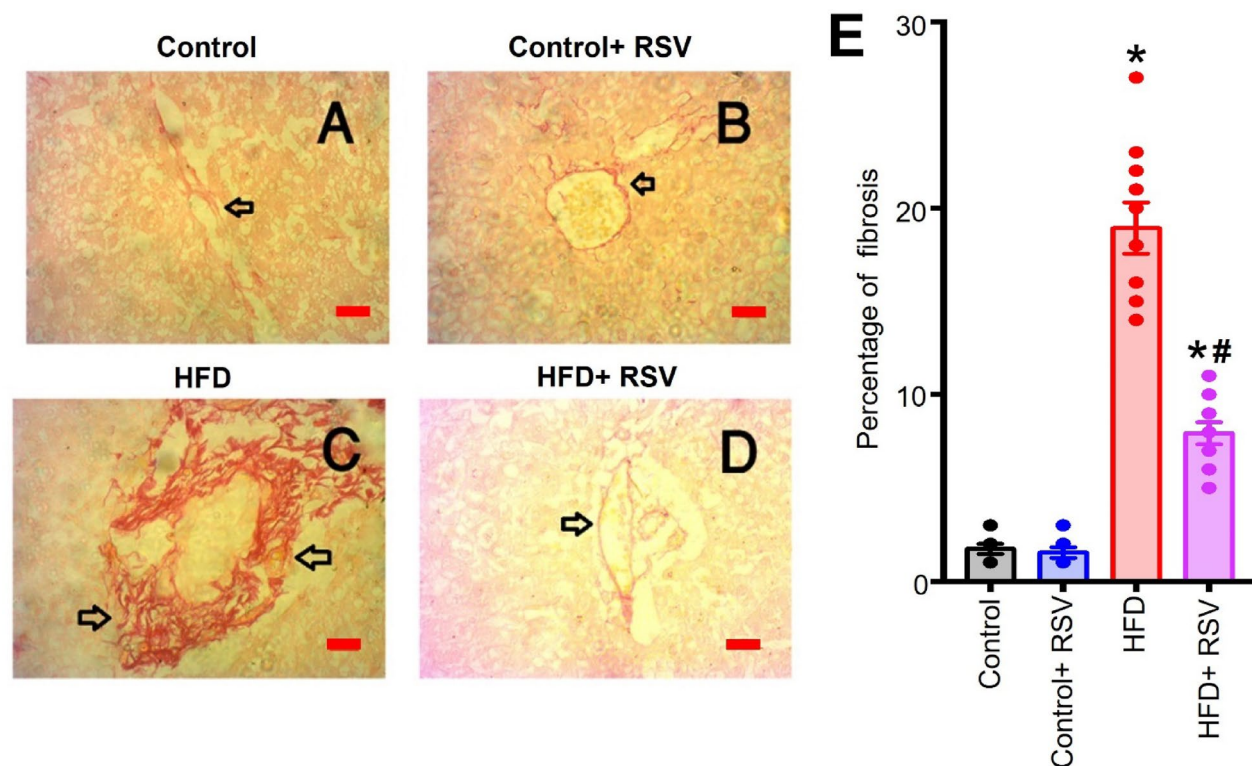
Fig. 10. Effect of resveratrol on collagen deposition and fibrosis in the liver of HFD-fed rats. (**A**) Control group: no collagen deposition observed in liver sections. (**B**) Resveratrol-treated control group: no collagen deposition observed. (**C**) High-fat diet (HFD) group: liver sections showed evident collagen deposition, indicating fibrosis. (**D**) HFD with resveratrol treatment: no collagen deposition observed in liver sections. Images were taken at 40× magnification. Scale bar in (**A**–**D**) 100 μm. (**E**) Mean data demonstrating resveratrol treatment significantly reduced hepatic fibrosis in HFD-fed rats. Statistical comparisons were made using one-way ANOVA followed by Tukey's post-hoc test. * $p < 0.05$ vs. Control, # $p < 0.05$ vs. HFD.

Discussion

This study provides new insight into the gut–liver axis as a mechanistic target of resveratrol therapy in the context of HFD-induced metabolic injury. Specifically, it investigated the effects of resveratrol, a natural polyphenol found in various food sources, on mitigating HFD-induced metabolic disturbances, particularly focusing on oxidative stress, inflammation, and tissue damage in the liver and intestine. The findings demonstrate that resveratrol treatment significantly suppresses HFD-induced liver dysfunction, weight gain, fat deposition, and inflammation, likely through its antioxidant and anti-inflammatory properties. Furthermore, resveratrol enhanced the expression of fat-metabolizing and antioxidant genes, inhibited hepatic steatosis, collagen deposition, and fibrosis, and importantly, preserved the mucus-producing goblet cell population in the intestine. The protection of goblet cells and the intestinal mucus barrier by resveratrol represents a novel finding that positions gut barrier integrity as a potential key mediator of its systemic metabolic benefits.

HFD is a major factor in the development of obesity, which is linked to insulin resistance and glucose intolerance[50]. In this study, HFD-fed rats showed significant weight gain, hyperglycemia, and glucose intolerance, consistent with previous research indicating that HFD can lead to these metabolic abnormalities[42,51]. Additionally, increased fat deposition in the peritoneal and mesenteric regions of HFD-fed rats was observed, contributing to decreased insulin sensitivity and fatty acid overflow to the liver, leading to steatosis[52]. Resveratrol treatment not only prevented weight gain but also improved glucose utilization and reduced fat accumulation, findings that align with previous studies demonstrating the anti-obesity effects of polyphenols and antioxidants[37,42,53]. The investigation revealed that HFD-fed rats had elevated plasma cholesterol levels, which were significantly reduced by resveratrol. These effects may involve not only resveratrol's known actions on lipid metabolism and inflammation but also its preservation of intestinal barrier integrity, which may limit endotoxin translocation into the blood and chronic systemic inflammation[53,54].

A key feature of HFD-induced liver damage is oxidative stress, which disrupts liver function and leads to the release of liver enzymes such as ALT, AST, and ALP into the bloodstream[55]. The study found a significant increase in these enzymes in HFD-fed rats, indicative of liver injury. Resveratrol treatment lowered the levels of these hepatic enzymes, suggesting that it reduced oxidative stress and ameliorated liver dysfunction[56]. This effect can be attributed to resveratrol's ability to enhance endogenous antioxidant defenses, as evidenced by the restoration of SOD, catalase, and GSH levels[37]. Reduced antioxidant enzyme activity in HFD-fed rats led to free radical accumulation and lipid peroxidation[57] which was mitigated by resveratrol, confirming its dual-
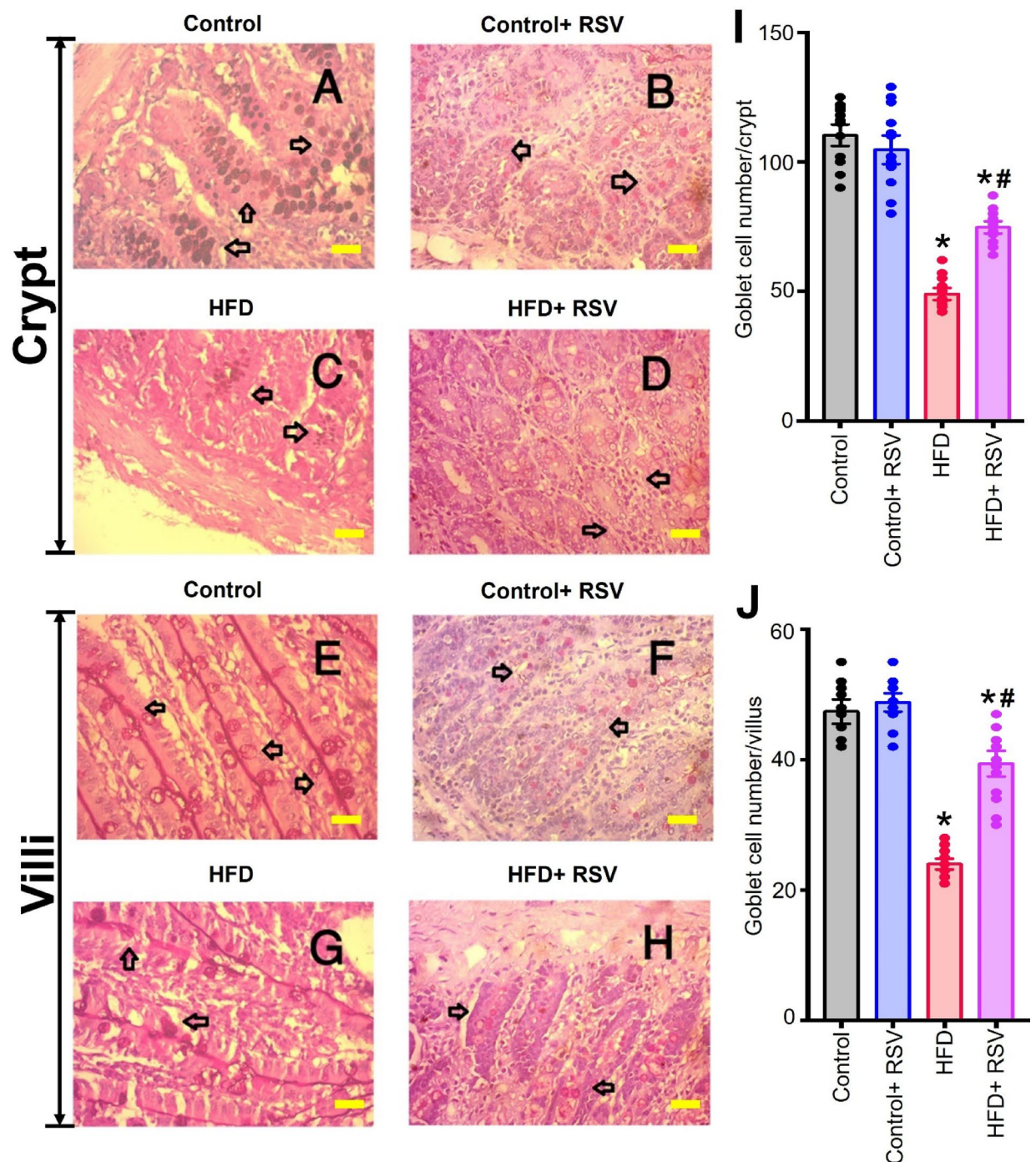
Fig. 11. Effect of resveratrol on goblet cell population in the crypt and villi regions of the intestine in HFD-fed rats. (**A,E**) Control group: normal goblet cell population observed in intestinal sections. (**B,F**) Resveratrol-treated control group: no change in goblet cell population observed. (**C,G**) High-fat diet (HFD) group: reduced goblet cell population observed in intestinal sections from both regions. (**D,H**) HFD with resveratrol treatment: restoration of goblet cell population observed in intestinal sections. Images were taken at 40x magnification. Scale bar in A-H 100 μm. (**I,J**) Mean data for the number of goblet cells per crypt and villus. * $p < 0.05$ vs. Control, # $p < 0.05$ vs. HFD.

action antioxidant role[58–60]. Furthermore, our data indicate that HFD-fed rats had significantly increased levels of oxidative stress markers, such as NO and APOP, alongside decreased expression of antioxidant genes like Nrf-2, SOD, and catalase. Resveratrol treatment preserved these antioxidant systems, suggesting that it improves the body's natural defenses against oxidative damage. Previous studies have shown that resveratrol can act as both a direct free radical scavenger[61] and a modulator of antioxidant gene expression through pathways involving

Nrf-2 activation[62]. The findings support this, as resveratrol not only reduced oxidative stress markers but also upregulated the expression of Nrf-2 and related antioxidant genes.

In terms of lipid metabolism, HFD increased the expression of pro-adipogenic transcription factors, including PPARγ, C/EBPα, and SREBP1c, which are key regulators of adipogenesis and lipid synthesis, and are upregulated in obese human and experimental models[63–65]. Resveratrol effectively inhibited the upregulation of these factors, thereby reducing fat accumulation in the liver and preventing hepatic steatosis[66]. This regulatory effect extended to glucose metabolism as well; resveratrol restored the expression of AMPK and adiponectin, both of which are crucial for glucose utilization and lipid oxidation[67,68]. Interestingly, while resveratrol improved AMPK and adiponectin levels, it did not significantly affect the HFD-induced downregulation of AdipoR1 and GLUT-4, suggesting that its effects on glucose transport require further investigation.

The anti-inflammatory effects of resveratrol were also evident in this study. HFD-fed rats displayed increased expression of pro-inflammatory cytokines such as IL-1, IL-6, TNF-α, and TGF-β, alongside elevated levels of NF-κB and iNOS, which drive inflammatory signaling[8]. Resveratrol treatment significantly reduced the expression of these markers, indicating its potent anti-inflammatory action. Additionally, histological analysis showed that resveratrol prevented hepatic steatosis, inflammatory cell infiltration, and collagen deposition, hallmarks of hepatic oxidative stress, inflammation, and fibrosis. Picrosirius red staining confirmed a marked reduction in fibrosis in resveratrol-treated rats, suggesting that resveratrol's combined antioxidant and anti-inflammatory effects contribute to its anti-fibrotic properties. Overall, the findings support earlier research by demonstrating the protective properties of resveratrol against liver disorders and its ability to reduce inflammation and hyperlipidemia.

One of the novel contributions of this study is the demonstration that resveratrol preserves the population of goblet cells in the intestinal lining of HFD-fed rats, maintaining the protective mucus barrier that is critical for gut epithelial integrity. Goblet cells are essential for mucin production, and their loss is associated with increased intestinal permeability and systemic endotoxemia[36]. This effect of resveratrol has not been previously described in HFD models and supports the concept that resveratrol's therapeutic potential extends beyond hepatic and mitochondrial effects to include protection of the gut barrier. This protection may play a pivotal role in limiting inflammation-driven liver injury. These findings align with and extend previous studies showing that resveratrol can improve gut health by maintaining epithelial integrity and supporting a balanced microbiome[69,70].

Conclusion

This study demonstrates that resveratrol mitigates HFD-induced metabolic dysfunction by reducing oxidative stress, inflammation, and tissue injury in both the liver and intestine. In addition to confirming its known effects on lipid metabolism and hepatic protection, our findings uniquely show that resveratrol preserves mucus-producing goblet cells and maintains intestinal barrier integrity, an underexplored mechanism in previous HFD studies. By protecting the gut–liver axis, resveratrol limits systemic inflammation and organ damage, offering a more integrated model of its therapeutic action. These results provide further support for resveratrol's multifaceted benefits and identify gut barrier preservation as a novel target for metabolic disease intervention. Future studies should explore long-term efficacy, human translation, and potential combinatorial therapies.

Data availability

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Author contributions

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Declarations

Competing interests

The authors declare no competing interests.

Ethics statement

The protocol was approved by the Ethics Committee for Animal Experiments in North South University (Protocol Number: AEC-006-2017).

Additional information

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