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Original Research

Rubus suavissimus S. Lee Extract Alleviates Oxidative Stress and Inflammation in H_2O_2-Treated Retinal Pigment Epithelial Cells and in High-Fat Diet-Fed Mouse Retinas

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Abstract

Background: Age-related macular degeneration (AMD) is the most common cause of visual disorders in the aged population and is characterized by the formation of retinal pigment epithelium (RPE) deposits and dysfunction/death of the RPE and photoreceptors. It is supposed that both oxidative stress and inflammation play a critical role in the pathogenesis of AMD. The development of therapeutic strategies against oxidative stress and inflammation in AMD is urgently needed. *Rubus suavissimus* S. Lee (RS), a medicinal plant growing in the southwest region of China, has been used as an herbal tea and medicine for various diseases. **Methods**: In this project, we evaluate the therapeutic potential of RS extract for AMD. We prepared RS extracts from dried leaves, which contained the main functional compounds. **Results**: RS extract significantly increased cell viability, upregulated the expression of antioxidant genes, lowered the generation of malondialdehyde and reactive oxygen species, and suppressed inflammation in H_2O_2-treated human RPE cells. In the *in vivo* study, treatment with RS extract attenuated body weight gain, lowered cholesterol and triglyceride levels in the liver and serum, increased antioxidant capacity, and alleviated inflammation in the retina and RPE/choroid of mice fed a high-fat diet. **Conclusions**: Our findings suggest that RS extract offers therapeutic potential for treating AMD patients.

Keywords: *Rubus suavissimus* S. Lee; age-related macular degeneration; retinal pigment epithelial cells; oxidative stress; inflammation; high-fat diet-fed mice

1. Introduction

Age-related macular degeneration (AMD), the most common visual disorder in humans over 50, currently affects approximately 200 million individuals worldwide [1]. The predominant clinical features of AMD include retinal pigment epithelium (RPE) atrophy and the presence of extracellular deposits (known as drusen) underneath the RPE. AMD is classified into early stage (drusen size of 63–125 µm without pigmentary abnormality), intermediate stage (drusen size $\geq$125 µm with/without pigmentary abnormality), and late stage (wet AMD with neovascularization and dry AMD with geographic atrophy) [2]. The dry form, accounting for approximately 90% of AMD cases, has no effective treatment; wet AMD, on the other hand, can be effectively treated with antibodies against vascular endothelial growth factor (VEGF) [2]. The development of effective treatment at the early or intermediate stages to stop or slow the progression to the late stage is urgently needed.

The retina has an extremely high oxygen consumption rate and produces high levels of reactive oxygen species (ROS), resulting in the pathogenesis of retinal degeneration, including AMD [3,4]. In AMD, the end-products of lipid peroxidation, for example, malondialdehyde (MDA) and carboxyethylpyrrrole (CEP), are associated with drusen formation and RPE dysfunction [5]. CEP-modified proteins are much more abundant in the plasma and the outer retinas of AMD patients than in those of controls [6,7]. Mice immunized with CEP adduct demonstrated AMD-like pathology [8]. ROS can also oxidize cholesterol to 7-ketocholesterol (7-KC), enriched in aged RPE cells and drusen. 7-KC has been shown to cause damage to human and rodent RPE cells. 7-KC can also enhance the expression of proinflammatory cytokines in RPE cells, which possibly contribute to AMD pathogenesis [9]. Accumulated evidence has shown that excessive generation of ROS induces nuclear factor-κB (NF-κB), mitogen-activated protein kinases (MAPK), and NLRP3 signaling pathways in RPE cells, resulting in the generation of proinflammatory factors, for example, TNF-α, IL-6, and IL-1β [4]. Oxidative stress also causes the breakdown of the retinal-blood barrier, a pathological feature of AMD [10]. Thus, targeting oxidative damage and inflammation is likely to benefit AMD patients.

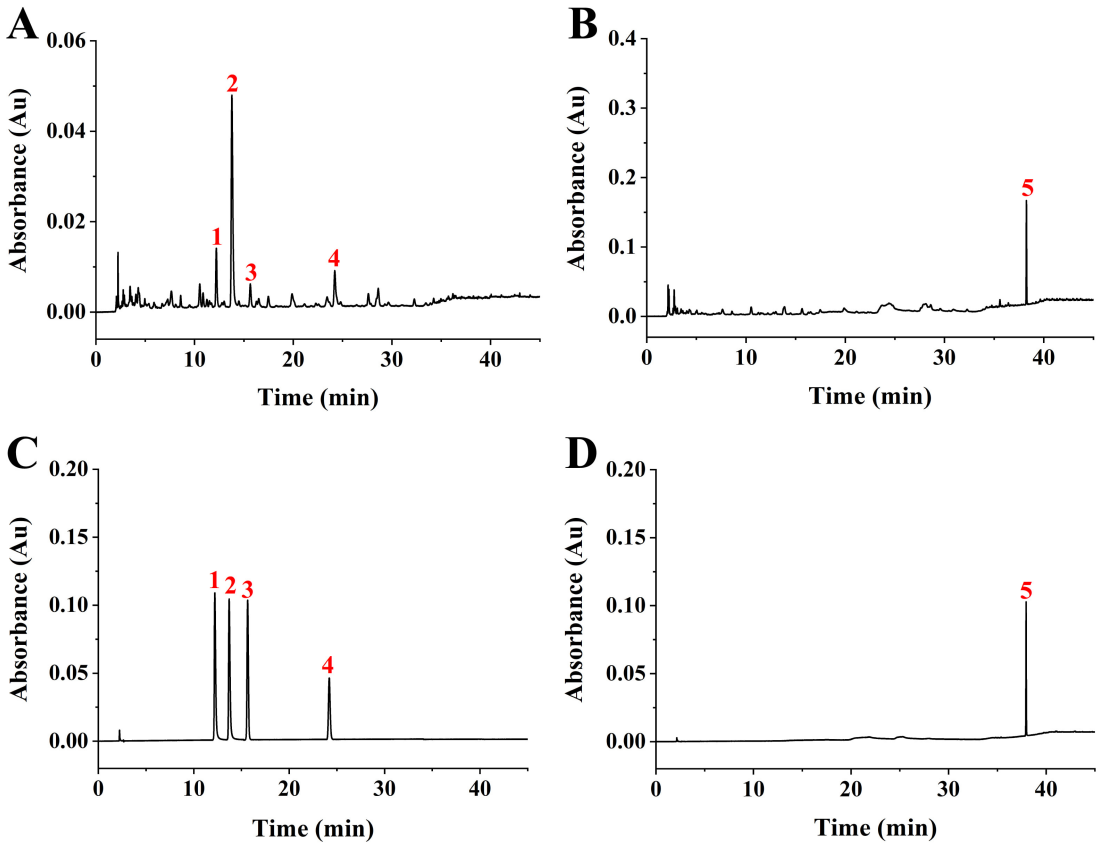

Fig. 1. High performance liquid chromatography (HPLC) analysis of *Rubus suavissimus* S. Lee (RS) extract. (A) HPLC chromatogram of the RS extract at 254 nm. (B) HPLC chromatogram of the RS extract at 210 nm. (C) HPLC chromatogram of standard compounds at 254 nm. 1, rutin; 2, ellagic acid; 3, hyperoside; 4, myricetin. (D) HPLC chromatogram of standard compounds at 210 nm. 5, rubusoside.

Rubus suavissimus S. Lee (RS) is widely distributed in Southwest China, particularly in Guangxi and Guizhou provinces [11]. The leaves of RS contain the natural sweetener rubusoside and have been used as a sweet tea by the local population. Different types of functional compounds, including polyphenols, flavonoids, diterpenes, lignans, and triterpenoids, have been identified from RS extracts [11]. Early studies have shown that RS extract promotes adipogenesis in preadipocytes by upregulating the expression of adipogenic transcription factors and their target genes [12]. RS extract has been reported to lower the level of blood glucose and to reduce both body weight gain and abdominal fat accumulation in rats fed a high-fat diet [13]. RS extract has also been shown to decrease lipid droplet formation in the livers of mice and hamsters fed a high-fat diet, possibly by regulating the proliferator-activated receptor/sterol regulatory-element binding protein (PPAR-SREBP) pathway [14,15]. Recently, Zhang *et al.* [16] reported that RS extract inhibited lipopolysaccharide (LPS)-induced chronic inflammation in mice by decreasing the production of proinflammatory factors, including MCP-1, IL-6, and TNF-α. RS extract has also been demonstrated to have antioxidant, antihypertensive, anticancer, and antidiabetic activities [17–19]. However, there are no studies investigating the effect of RS extract on retinal disorders.

In this study, we preliminarily characterize the chemical constituents of RS extract and evaluate the protective capacities of RS extract against oxidative stress and inflammation in H_2O_2-exposed human RPE cells and in the retinas of high-fat diet-fed mice.

2. Materials and Methods

2.1 Identification of Chemical Constituents in RS Extract

A total of 200 g dried RS leaves were ground and soaked in 90% ethanol for 3 h. The liquid extract was filtered and dried to obtain 30.6 g of powder. Constituents of RS extract were identified using high performance liquid chromatography (HPLC) analysis using a Waters XBridge® BEH C18 column (150 mm × 3.0 mm, 2.5 µm, Waters, Framingham, MA, USA) with a Waters ACQUITY UPLC H-Class instrument. The mobile phase was composed of (A) acetonitrile (CH_3CN) Merck, Germany) and (B) phosphoric acid (Sigma-Aldrich, Germany) in water (0.1%, v/v), and the gradient elution consisted of 15% A at 0 min, 25% A at 30 min, 60% A at 40 min, and 100% A at 45 min with a flow rate of 0.3 mL/min at 30 °C. The

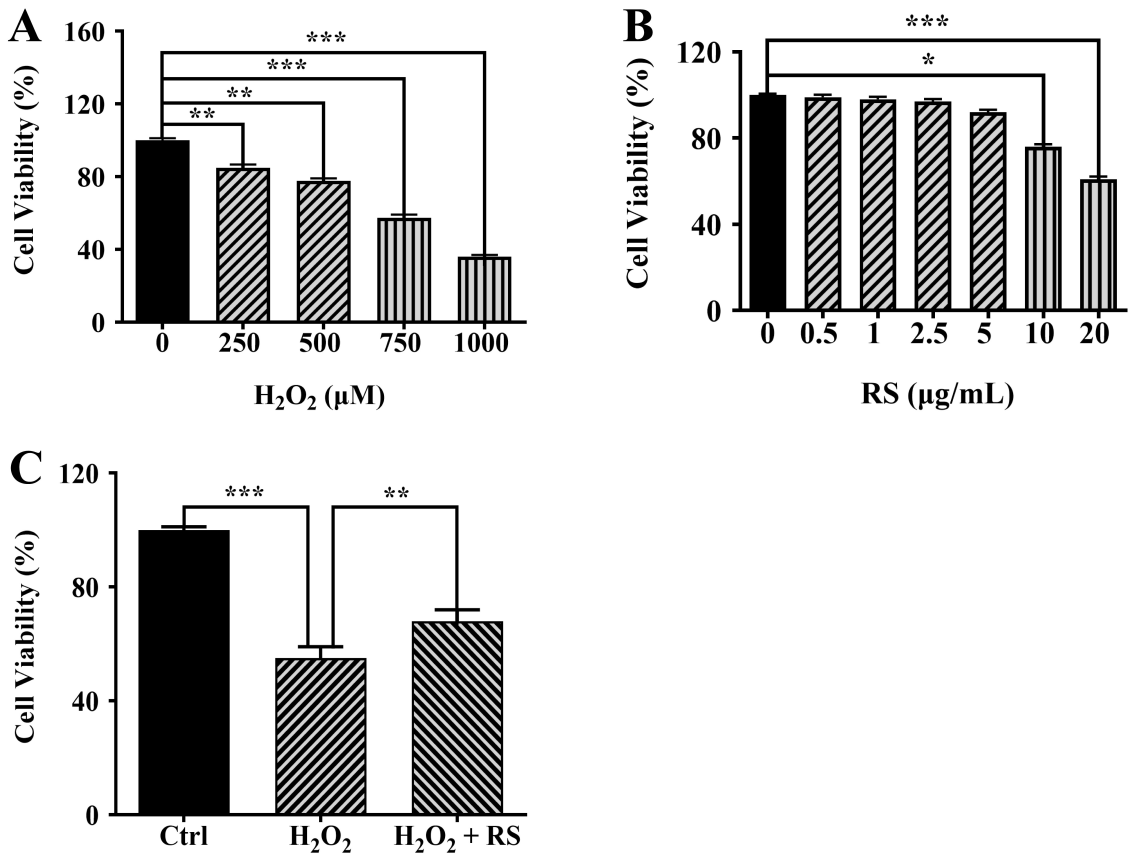

Fig. 2. RS reduces H_2O_2-induced cytotoxicity. (A) H_2O_2 treatment resulted in a significant decrease in cell viability. (B) Effect of RS extract on the viability of human retinal pigment epithelium (ARPE-19) cells treated with different concentrations of RS extract for 24 h. (C) Effect of RS extract on H_2O_2-induced cytotoxicity in ARPE19 cells. Data were analyzed using one-way ANOVA and a Tukey's post hoc test and displayed as the mean ± SEM (n = 3). *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

photodiode array detector was operated at 210 nm and 254 nm. The volume of injected samples was 2.0 µL.

2.2 Cell Viability Assay

Human RPE (ARPE-19) cells were seeded in 96-well plates (5×10^4 cells/well) in DMEM/F12 medium (Cat No 11330032, Gibco, Thermo Fisher Scientific, Waltham, MA, USA) supplemented with penicillin and streptomycin (50 IU/mL) and were incubated for 20 h. The cells were exposed to H_2O_2 or RS extract or a combination of H_2O_2 and RS extract for 24 h. The medium was discarded, and the cells were washed with phosphate buffer saline (PBS) (Cat No BL302A, Biosharp, Beijing, China) twice. Cell viability was examined using an 3-(4,5-dimethlythiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) (Cat No 1334MG250, BioFroxx, Germany) assay guided by an early description [20].

2.3 Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

Total RNA was extracted from control and treated ARPE-19 cells using TRIzol Reagent® (15596-018, Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. cDNAs were synthesized with a commercial cDNA synthesis kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China) according to the manufacturer's protocol. The expression of target genes was measured using a real-time PCR kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China) as described by the manufacturer and calculated using the $2^{-\Delta\Delta ct}$ formula. The sequences of the primers used for qRT-PCR are shown in **Supplementary Table 1**.

2.4 Animal Experiments

Animal experiments were approved by the Hunan University of Chinese Medicine Animal Ethics and Welfare Committee (SYXK (Xiang) 2019-0009). C57BL/6J mice aged four weeks (50% male and 50% female) were housed in the Hunan University of Chinese Medicine Animal Unit. All mice were randomly divided into three groups (n = 8 per group); one group was fed a normal diet (ND), whereas the other two groups were fed a high-fat diet (HFD). Normal diet (Cat No. D12450B) and high-fat diet (Cat No. D12492, 78.75% normal diet, 10% corn oil, 1% cholesterol, 10% lard, and 0.25% sodium cholate) were purchased from Research Diets Inc. After 12 weeks, the ND group and

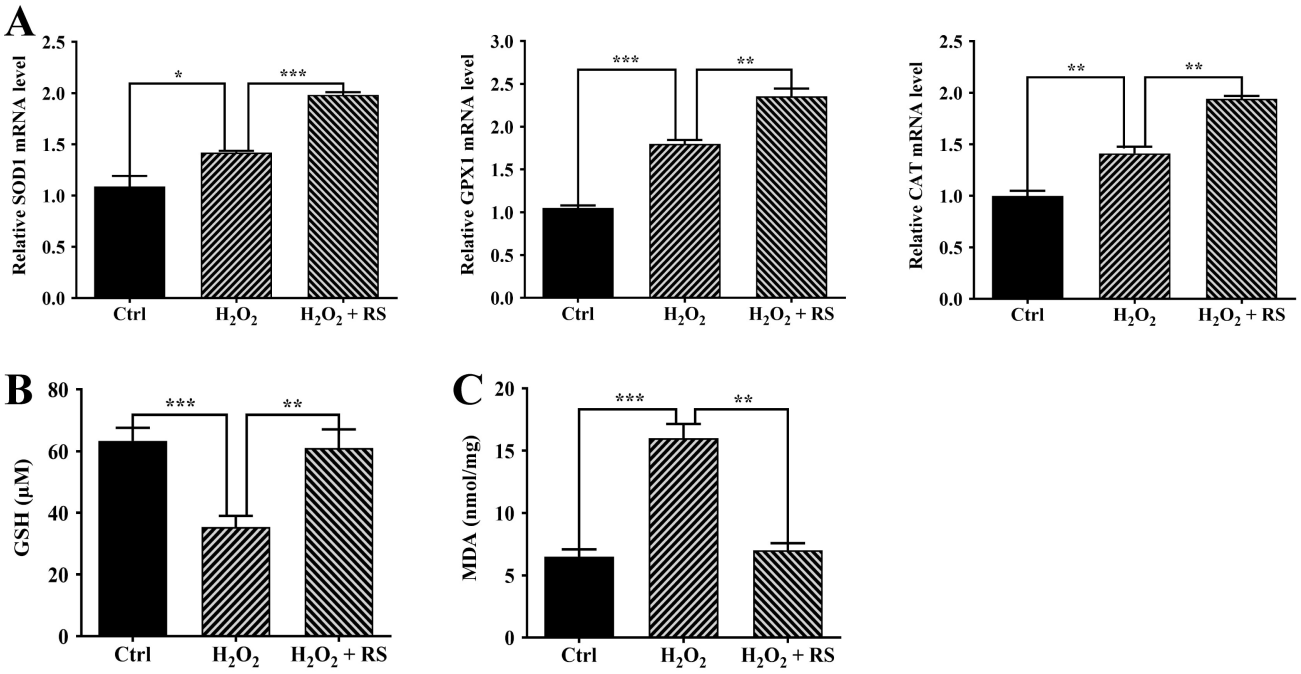

Fig. 3. Increased antioxidant capacity in retinal pigment epithelium (RPE) cells treated with RS extract. (A) *SOD1*, *GPX1*, and *CAT* expression was measured by quantitative real-time polymerase chain reaction (qRT-PCR). (B) The basal glutathione (GSH) levels in untreated (Ctrl) and treated ARPE-19 cells. (C) Malondialdehyde (MDA) levels in untreated (Ctrl) and treated ARPE-19 cells. Data were analyzed using one-way ANOVA and a Tukey's post hoc test and displayed as the mean ± SEM (n = 3). *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

one HFD group received intragastric treatment of sterilized saline daily for four weeks; the other HFD group (termed the HFD + RS group) received intragastric administration of RS extract dissolved in physiological saline (350 mg/kg/d) daily for 4 weeks. The ND group was fed a normal diet during the treatment period, whereas the HFD and HFD + RS groups were fed a high-fat diet. Animals were sacrificed after the treatment, and tissues were collected and kept in a –80 °C freezer until further analysis.

2.5 Biochemical Assays

The levels of total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglyceride (TG) in mouse sera were measured using an automatic biochemical analyzer (HITACHI 7600, Hitachi HighTech Co., Ltd., Tokyo, Japan).

The superoxide dismutase activity, catalase activity, glutathione level, and malondialdehyde level in ARPE-19 samples and mouse samples were quantified using commercial kits (Cat No. A001-3-2, SOD; Cat No. A007-1-1, CAT; Cat No A006-1-1, GSH; and Cat No. A003-4-1, MDA; Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China) based on the manufacturer's instructions.

2.6 Enzyme-Linked Immunosorbent Assay (ELISA)

The levels of TNF-α, IL-6, and IL-1β in ARPE-19 cell culture media, mouse sera, and mouse tissue lysates were determined using commercial kits (Cat No. H002, IL-1β; Cat No. H007-1-1, IL-6; and Cat No. H052-1, TNF-α; Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China) guided by the manufacturer's protocols.

2.7 Statistical Data Analysis

One-way or two-way analysis of variance (ANOVA) was applied to analyze the data, presented as the mean ± standard error of the mean (SEM). $p < 0.05$ was considered significant.

3. Result

3.1 Identification of the Main Components in the RS Extract

The RS extract was subjected to HPLC analysis along with five standards: rutin, ellagic, hyperoside, myricetin, and rubusoside. Based on the five standards, we found that ellagic acid and rubusoside were the major compounds in the RS extract (Fig. 1), which was in agreement with previous reports [10,16]. The three other compounds, rutin, hyperoside, and myricetin, were also detected in the RS extract at low levels. The retention times for ellagic acid and rubusoside were 13.78 min and 37.94 min, respectively.

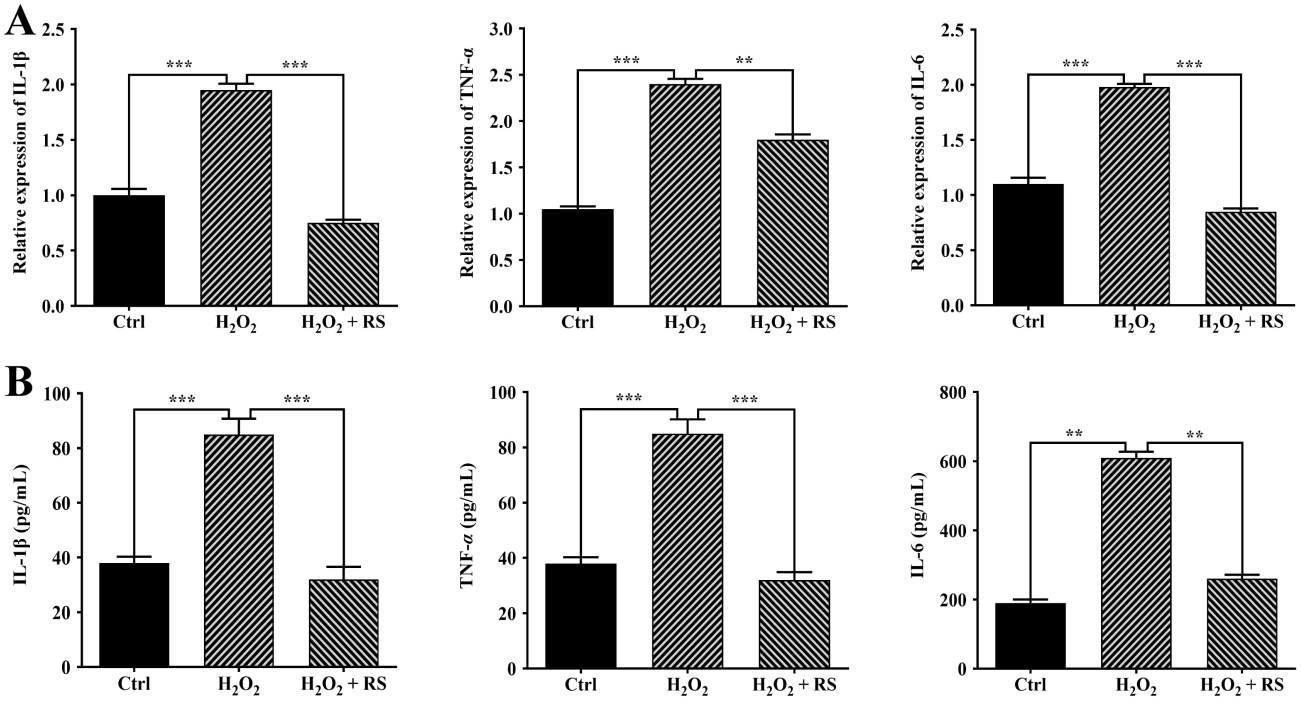

Fig. 4. Effects of RS extract on inflammation in H_2O_2-treated ARPE-19 cells. (A) The expression of IL1β, IL6, and TNF-α in untreated (Ctrl) and treated ARPE-19 cells was examined using qRT-PCR. (B) The levels of secreted IL1β, IL6, and TNF-α from untreated (Ctrl) and treated cells were measured using an ELISA. Data were analyzed using one-way ANOVA and Tukey's post hoc test and displayed as the mean $\pm$ SEM (n = 3). $^{**}p < 0.01$; $^{***}p < 0.001$.

The linear regression equations based on the concentration (X, µg/mL) and the peak area (Y) for ellagic acid and rubusoside were Y = 39.9951X + 14.849 (coefficient, 0.9999) and Y = 26.1295X – 8.697 (coefficient, 0.9997), respectively. The contents of ellagic acid and rubusoside in RS leaves were 26.82 mg/g and 34.23 mg/g, respectively.

3.2 RS Treatment Enhanced Cell Viability in H_2O_2-Treated Human RPE Cells

First, we treated ARPE-19 cells with H_2O_2 at 250, 500, 750, and 1000 µM for 24 h and measured cell viability. We found that H_2O_2 treatment caused a marked decrease in cell viability in ARPE-19 cells compared to untreated cells (Fig. 2A). We also assessed whether RS extract was toxic to ARPE-19 cells by treating the cells with RS extract at 0.5, 1.0, 2.5, 5.0, 10 and 20 µg/mL for 24 h and determining cell viability. Treatment with RS extract at 0.5, 1.0, 2.5, and 5.0 µg/mL did not cause a significant change in cell viability compared to untreated cells; however, 10 and 20 µg/mL RS extract caused a significant decrease in cell viability (Fig. 2B). Based on the data, we chose 750 µM H_2O_2 and 5.0 µg/mL RS extract for all subsequent experiments. When ARPE-19 cells were cotreated with 750 µM H_2O_2 and 5.0 µg/mL RS extract for 24 h, cell viability significantly increased by 62.00% compared to cells exposed to H_2O_2 alone (Fig. 2C).

3.3 RS Extract Increased Antioxidant Capacity in H_2O_2-Exposed RPE Cells

Our previous study has shown that H_2O_2 induces oxidative damage in ARPE-19 cells and that the natural products, gypenosides, protect RPE cells from this damage [20]. Here we investigated whether RS extract had a similar effect by initially examining the mRNA levels of antioxidant genes (*SOD1*, *GPX1*, and *CAT*) in ARPE-19 cells treated with H_2O_2 or RS + H_2O_2. We found that antioxidant gene expression was significantly upregulated in H_2O_2-treated cells compared to untreated cells, possibly due to H_2O_2-induced self-defense; treatment with RS extract further significantly enhanced the expression of antioxidant genes compared to that of cells exposed to H_2O_2 only (Fig. 3A). H_2O_2 treatment of cells resulted in markedly decreased basal glutathione (GSH), whereas RS extract reversed these H_2O_2-induced effects (Fig. 3B). We found that H_2O_2 exposure markedly increased MDA production, compared to untreated controls; cotreatment with RS extract significantly lowered MDA levels compared to that of cells incubated with H_2O_2 alone (Fig. 3C).

3.4 RS Extract Inhibits H_2O_2-Induced Inflammation in RPE Cells

Our previous study has demonstrated that H_2O_2 administration induces inflammation in ARPE-19 cells [20]. RS extract has been shown to decrease the production of proinflammatory cytokines [16]. In this study, we treated

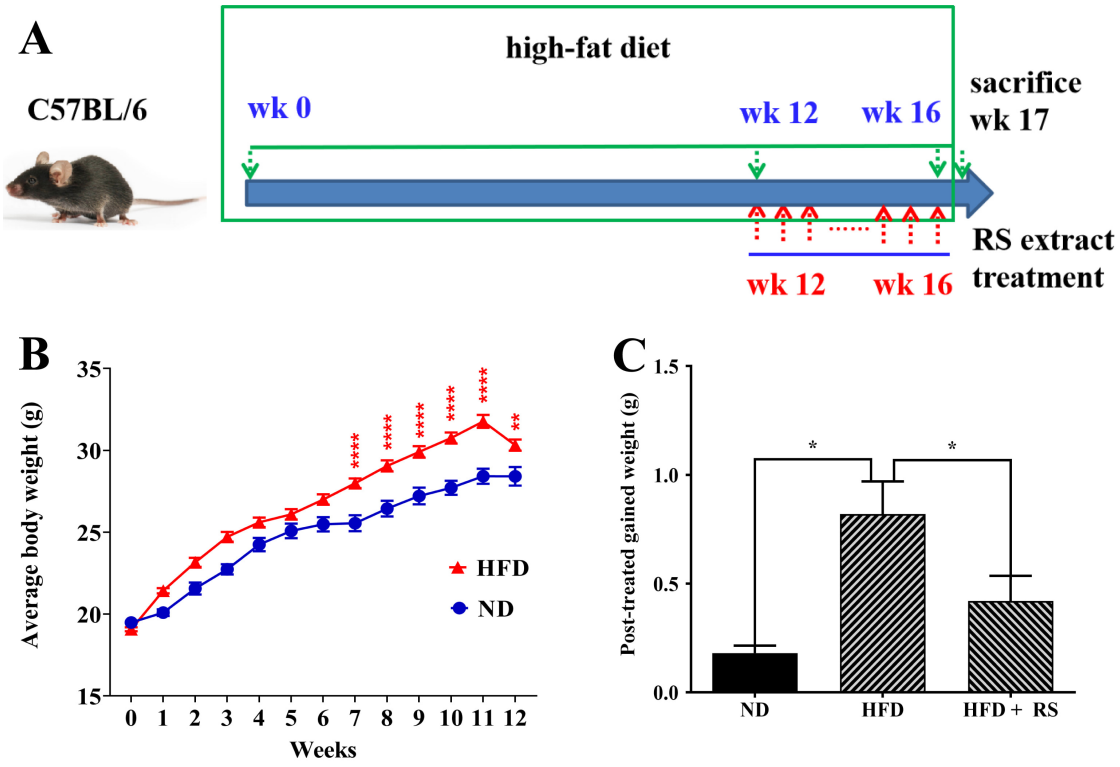

Fig. 5. RS extract reduced body weight gain. (A) Schematic of the treatment plan. (B) Changes in body weight of C57BL/6 mice fed a normal diet (ND) and a high-fat diet (HFD) during the experimental period. (C) Effects on body weight gain among the ND, HFD, and HFD + RS groups. (B) Data were analyzed using two-way ANOVA and a Bonferroni test or (C) using a one-way ANOVA and Tukey's post hoc test and presented as the mean ± SEM. *$p < 0.05$, **$p < 0.01$, ****$p < 0.0001$.

ARPE-19 cells with 750 μM H_2O_2 or 750 μM H_2O_2 + 5.0 μg/mL RS for 24 h; compared with untreated cells, the transcript levels of IL-1β, IL-6 and TNF-α in ARPE-19 cells treated with H_2O_2 only were significantly increased, whereas cotreatment with RS extract markedly reduced the expression of these proinflammatory genes (Fig. 4A). We also measured IL-1β, IL-6, and TNF-α secretion in cell culture using an enzyme-linked immunosorbent assay (ELISA); compared to that in the untreated cell group, the production of IL-1β, IL-6, and TNF-α was significantly higher in the H_2O_2-treated group, whereas the H_2O_2 + RS group had significantly lower secretion of these proinflammatory factors (Fig. 4B). These results demonstrate that treatment with RS extract reversed H_2O_2induced cytokine expression.

3.5 RS Extract Ameliorated Body Weight Gain and Mediated Lipid Production in the Liver and Serum of High-Fat Diet-Fed Mice

To determine the function of RS extract *in vivo*, we established an obese mouse model. Animals were fed with ND or HFD for 12 weeks. Then, the ND group and one HFD group were administered a vehicle for four weeks, whereas the other HFD group received treatment with RS extract (HFD + RS). During the treatment phase, these animals were fed either ND (ND group) or HFD (HFD group and HFD + RS group) (Fig. 5A). Body weight was monitored during the whole experimental process. High-fat diet-fed mice showed significant weight gain after seven weeks of feeding compared to animals fed a normal diet (Fig. 5B). Administration of RS extract significantly limited weight gain in the high-fat diet-fed animals compared to the animals fed a high-fat diet alone (Fig. 5C); this is in agreement with a previous report [13].

High-fat diet consumption can enhance lipid accumulation in the liver, causing increased liver weight and damage to the liver. The liver index is an important indicator of obesity in rodents. The HFD mice had markedly increased liver weight compared to the ND mice; treatment with RS resulted in a notable decrease in liver weight, although this was not significant when compared to the HFD mice (Fig. 6A,B). However, the liver index of the HFD animals was significantly higher compared to that of the ND animals. After treatment with RS extract, the index was notably decreased compared to that of the HFD groups (Fig. 6C).

A high-fat diet is expected to affect animal lipid metabolism. We first measured total cholesterol and glyceride in the liver of the three groups and found that a high-fat diet induced a significant increase in the total cholesterol and glyceride levels in the liver compared to that of mice fed a normal diet; treatment with RS extract reversed

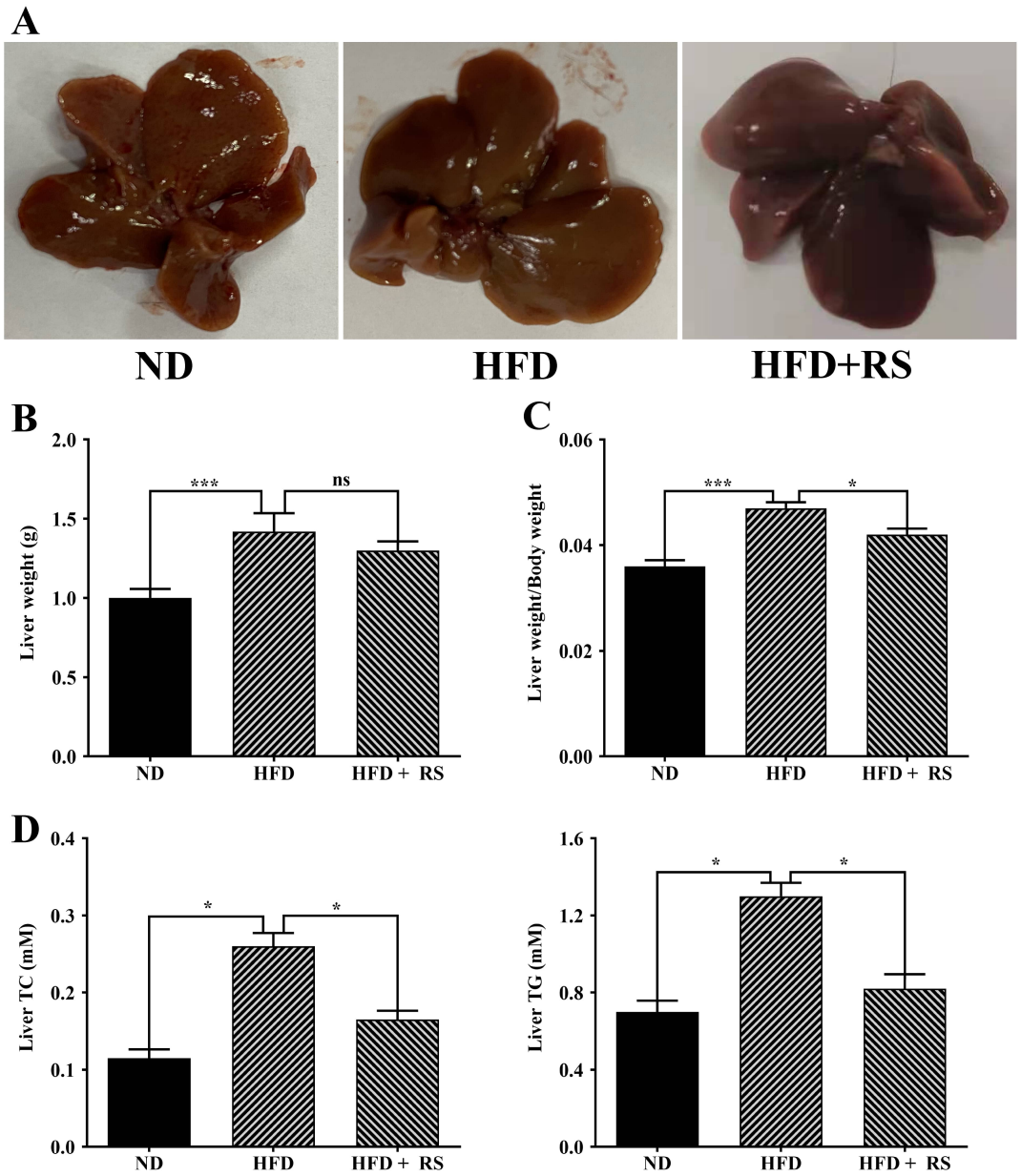

Fig. 6. Treatment with RS extract decreased the liver index and the levels of total cholesterol and triglycerides. (A) Images of livers from mice fed with ND, HFD, or HFD with RS extract (HFD + RS). (B) Liver weights of the three groups. (C) Liver weight/body weight (liver index) of the three groups. (D) Measured total cholesterol and triglycerides. ns, no significance; *$p < 0.05$; ***$p < 0.001$.

the high-fat diet-induced effect (Fig. 6D). We also examined cholesterol, triglyceride, LDL-cholesterol and HDL-cholesterol in mouse serum. Total cholesterol, triglyceride, and LDL-cholesterol were markedly higher in the serum of high-fat diet-fed animals, compared to animals fed with a normal diet, whereas RS extract-treated animals had a significant decrease in these lipids, compared to concentrations in high-fat diet-fed animals. HDL-cholesterol levels were significantly lowered in the serum of mice fed a high-fat diet, an effect that was reversed as a result of treatment with RS extract (Fig. 7).

3.6 RS Ameliorated HFD-Induced Oxidative Stress in Mice

Accumulated evidence has demonstrated that obesity is associated with oxidative stress [21–23]. We found a higher production of ROS, a significant decrease in Superoxide Dismutase (SOD) activity and GSH levels, and a marked increase in the MDA content in the RPE/choroid and retina of HFD-fed mice compared to ND-fed mice. Treatment with RS extract counteracted the high-fat diet-induced effects (Fig. 8).

Further investigation showed that high-fat diet-fed animal serum also had significantly higher levels of ROS and MDA, decreased SOD activity, and low levels of GSH

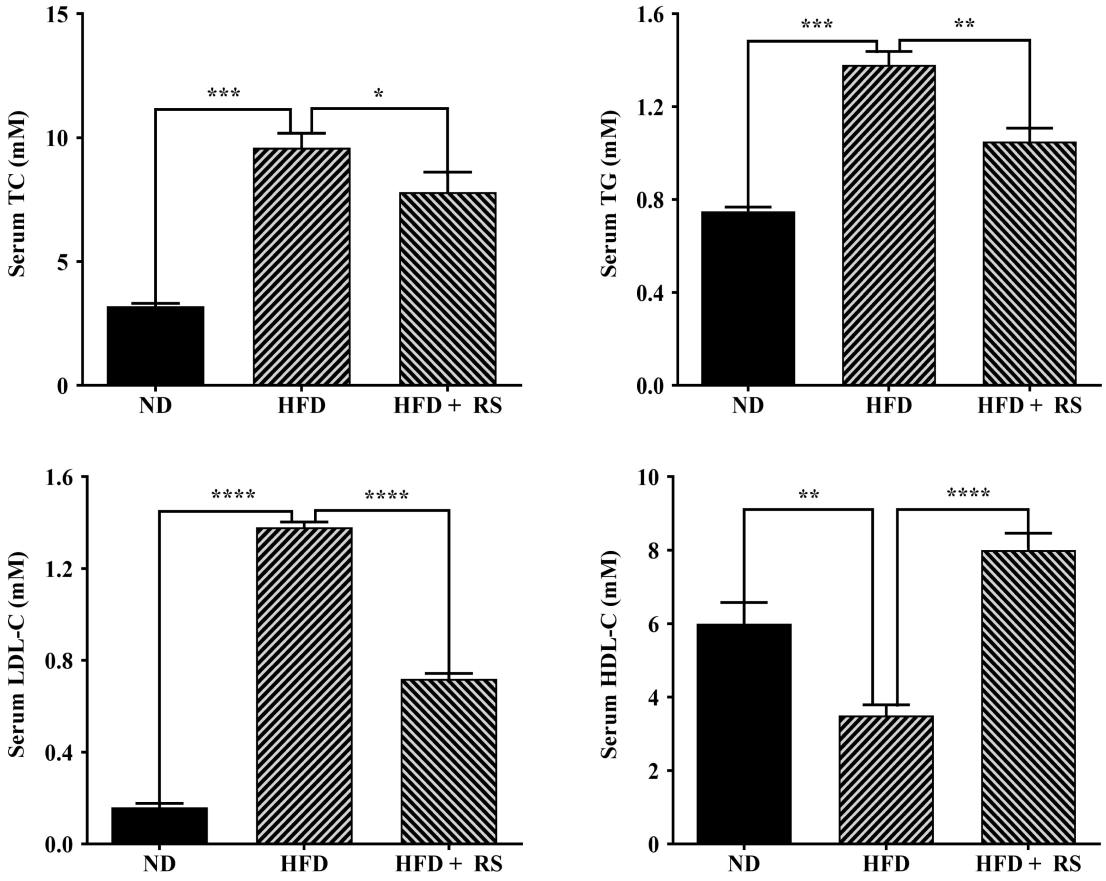

Fig. 7. Effects of RS extract on lipids in the serum of mice fed a high-fat diet. The levels of total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) in the serum of mice fed ND, HFD or HFD and treated with RS extract (HFD + RS). Data were analyzed using one-way ANOVA and Tukey's post hoc test and displayed as the mean ± SEM. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$.

compared to those of animals fed a normal diet. In RS extract-treated animal serum, ROS production was notably decreased, SOD activity and GSH levels were significantly increased, and MDA was markedly decreased compared to that of animals fed with HFD alone (Fig. 9).

3.7 RS Attenuated Inflammation Induced by a High-Fat Diet

Growing evidence indicates that obesity can activate inflammatory signaling pathways, such as the NF-κB pathway, which can induce the production of proinflammatory cytokines [23,24]. We found that the production of the proinflammatory mediators IL-1β, IL-6, and TNF-α was significantly increased in the RPE/choroid and retinas of mice fed with HFD compared to that of mice fed with ND; treatment with RS extract significantly suppressed the production of these cytokines (Fig. 10A,B). Similarly, these three inflammation factors were markedly higher in the serum of animals fed a high-fat diet than in animals fed a normal diet; RS extract reversed these high-fat diet-induced effects (Fig. 10C).

4. Discussion

In this study, we investigated the protective effects of RS extract against oxidative stress and inflammation in H_2O_2-treated human RPE cells and obese mice. Our results demonstrated that RS extract counteracted H_2O_2-induced oxidative damage and inflammation in RPE cells and attenuated high-fat diet-stimulated oxidative stress and inflammation in mouse RPE/choroid, retina, and serum. Oxidative stress and inflammation are thought to contribute to the pathogenesis of complex retinal disorders, such as AMD, diabetic retinopathy, and glaucoma [25–27]; oxidative stress and inflammation are also involved in the development of inherited retinal diseases [28]. Therefore, the data suggest that RS extract might have therapeutic potential for complex and inherited retinal disorders.

A recent study identified 69 compounds from RS extract, including gallic acid, ellagic acid, rubusoside, kaempferol, rutin, and caffeic acid, possibly mediating lipid metabolism [11]. Based on the standards, we identified five predominant compounds: ellagic acid, rubusoside, rutin, myricetin, and hyperoside. The roles of these compounds in cellular function and diseases have been widely inves-

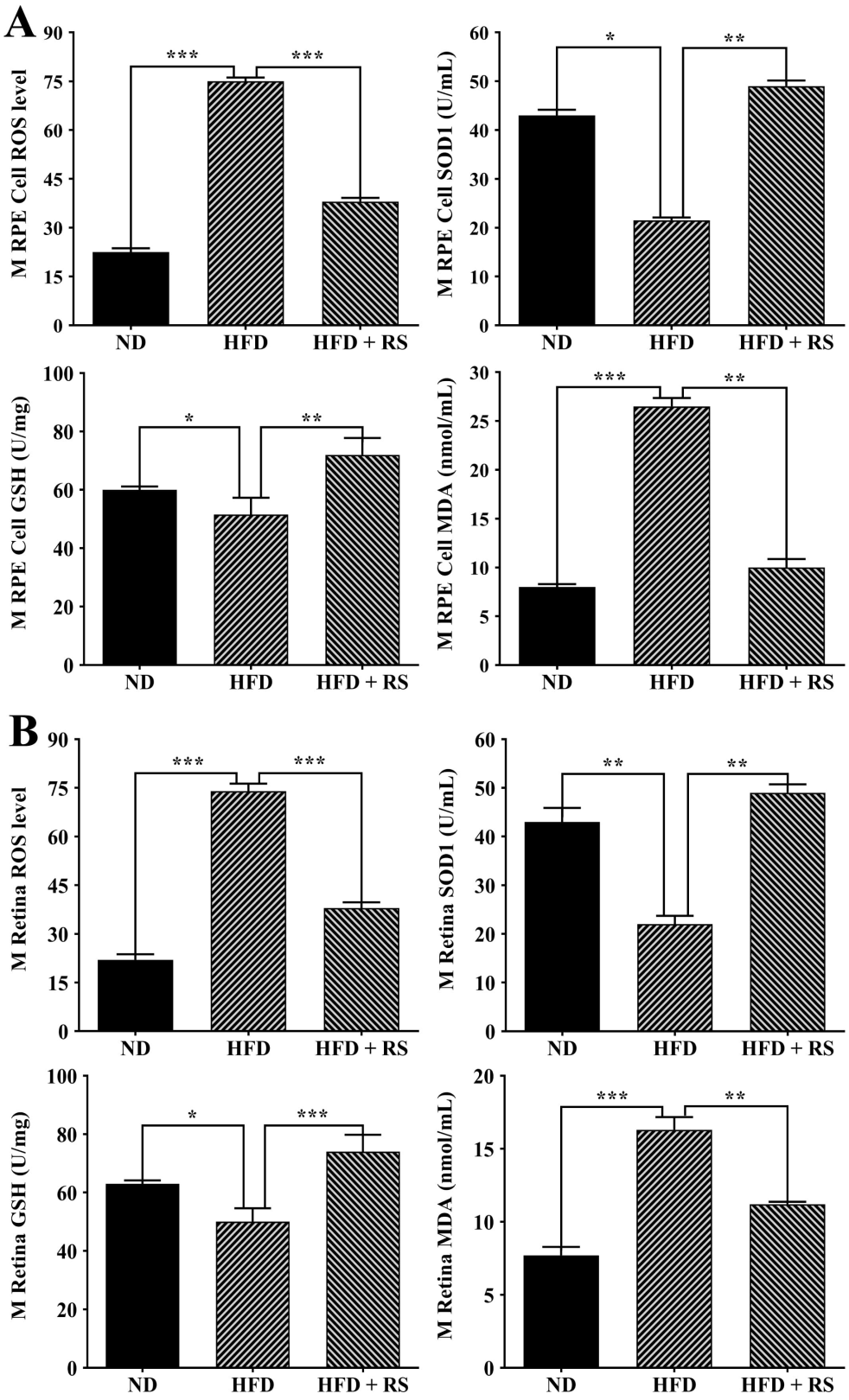

Fig. 8. RS extract treatment enhanced the antioxidant capacity of the RPE/choroid (A) and retinas (B) of mice fed a high-fat diet. The levels of reactive oxygen species (ROS), GSH, and malondialdehyde (MDA) and SOD activity were measured in RPE/choroid and retinas of ND, HFD, and HFD + RS mice. Data were analyzed using one-way ANOVA and Tukey's post hoc test and expressed as the mean ± SEM. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

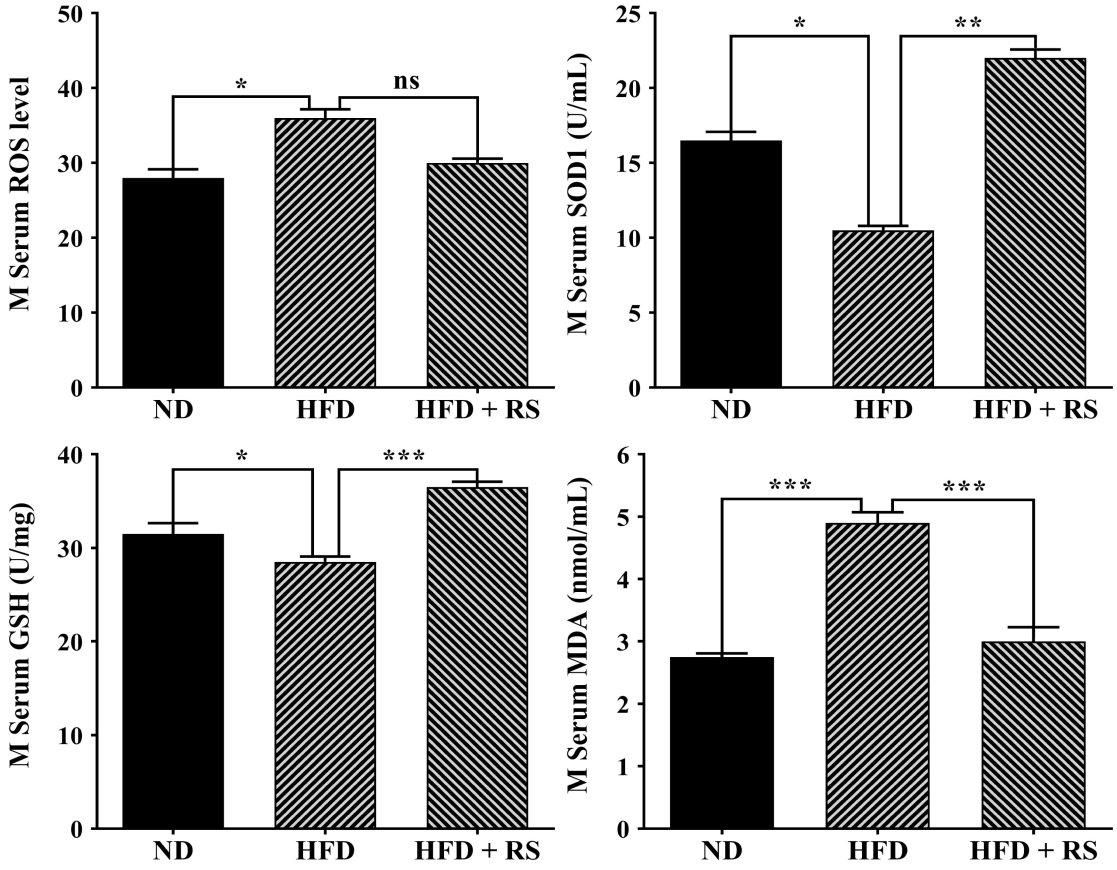

Fig. 9. RS extract mediated redox homeostasis in mouse sera. The levels of ROS, GSH, and MDA and the SOD activity were detected in the serum of mice fed a normal diet (ND), high-fat diet, or high-fat diet with concurrent treatment with RS extract. Data were expressed as the mean ± SEM and analyzed using one-way ANOVA and Tukey's post hoc test. ns, no significance; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

tigated. Ellagic acid (EA) has demonstrated a wide range of activities, particularly antioxidant and anti-inflammatory activities [29]. EA contains four hydroxyl groups that can scavenge both superoxide and hydroxyl groups. EA has been shown to inhibit oxidative stress and inflammation in chemical-induced neurodegenerative disorders [29]. Dietary EA inhibited inflammation and attenuated retinal pathology in a diabetic retinopathy rat model [30]. Rubusoside has been shown to enhance antioxidant enzyme activities, inactivate the NF-κB signaling pathway, decrease the production of inflammatory factors, and inhibit airway inflammation in an ovalbumin-induced mouse asthma model [31]. However, there is no research on rubusoside in retinal disorders. Rutin is also a well-studied compound that exhibits antioxidant and anti-inflammatory capacity and has been shown to upregulate the expression of antioxidant enzymes and decrease the expression of proinflammatory cytokines in neurodegenerative disorder models [32]. A previous study showed that rutin protects ganglion cells from hypoxia, excessive glutamate, or oxidative stress-induced damage [33]. Recent studies have shown that rutin protects RPE cells from tert-butyl hydroperoxide-induced oxidative injury by activating the NRF2 and ERK1/2 signaling pathways [34]. Rutin treatment also increased the antioxidant capacity and inhibited inflammation in rat diabetic retinas [35,36]. Myricetin also suppresses oxidative stress and neuroinflammation in central nervous system disorders by activating the NRF2, ERK1/2, AKT, CREB, and BDNF signaling pathways [37]. An early study reported that myricetin protected photoreceptors against A2E- or blue light-induced cell death in bovine primary retinal culture [38]. A recent study showed that myricetin inhibited acute bright light-induced ROS production, downregulated the expression of inflammation genes, and preserved photoreceptors in *Abca4* and *Rdh8* double-knockout mice [39]. Similarly, hyperoside protects against oxidative stress and inflammation in various diseases by regulating the NRF2 and NF-κB signaling pathways [40,41]. Hyperoside has been shown to decrease the production of ROS and MDA and increase SOD activity and cell viability in rat retinal vascular endothelial cells treated with high glucose; hyperoside also arrests retinal pathology in diabetic rats [42]. Therefore, the protective role of RS extract against oxidative stress and inflammation is possibly a function of these predominant compounds.

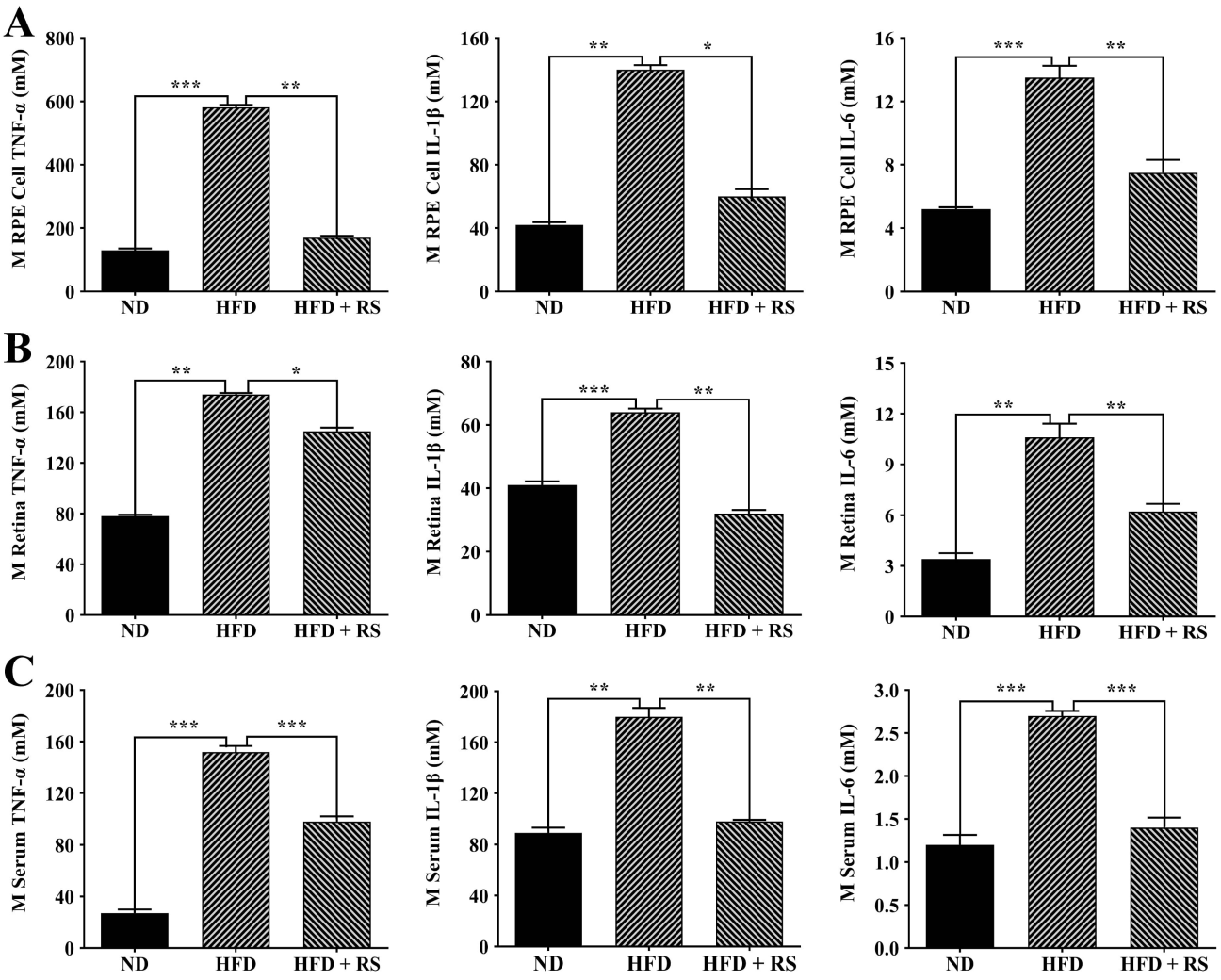

Fig. 10. Effect of treatment with RS extract on the inflammatory cytokines TNF-α, IL-1β and IL-6 in (A) RPE/choroid, (B) retina, and (C) serum of mice fed with ND, HFD, or HFD with RS extract treatment (HFD + RS). The data were analyzed using one-way ANOVA and Tukey's post hoc test (n = 6) and presented as the mean $\pm$ SEM. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Obesity is associated with late AMD and is possibly involved in the progression of AMD [43]. Genome-wide association studies have shown that variants in lipid homeostasis genes (*ABCA1*, *CETP*, *APOE*, and *LIPC*) are associated with AMD [44]. High-fat/cholesterol diets also result in AMD-like retinal pathology in animals [45,46]. Excessive lipids taken up from the intestine in a high-fat diet are stored in the liver or accumulate in peripheral tissues via blood circulation, which results in systemic and local oxidative stress and inflammation [24]. Previous investigations have demonstrated that high-fat diets caused significantly increased levels of cholesterol and glyceride in mouse liver, serum, retina, and RPE/choroid; high-fat diet-fed mice also had markedly increased levels of proinflammatory cytokines in sera, retina, and RPE/choroid [22,23]. In the current study, a high-fat diet also had similar effects on lipids, oxidative stress, and inflammation, whereas RS extract counteracted these effects. Other researchers have reported that RS extract decreased the levels of serum cholesterol and triglycerides in rats and hamsters fed a high-fat diet [13,15]. In fact, individual compounds identified in RS extract have been shown to mediate lipid metabolism. For example, rubusoside mediates lipid metabolism in hamsters fed a high-fat diet [47]. Recently, researchers have begun to investigate the underlying mechanisms of lipid metabolism mediation by RS extract. The prediction by Jiang *et al.* [11,15] that the effects of RS extract on lipid homeostasis may be associated with 20 signaling pathways, including the PPAR/SREBP pathway, has been experimentally confirmed. The effect and molecular mechanisms of RS extract on lipid metabolism in retina and RPE/choroid require further investigation.

VEGF is a critical player in the development of wet AMD, and anti-VEGF antibodies are commonly utilized in wet AMD treatment [2]. Oxidative stress can upregulate VEGF expression in human RPE cells [48]. A high-fat diet also increases VEGF expression in retina and RPE/choroid of rodents [49,50]. RS extract has been shown to atten-

uate VEGF expression in preadipocytes, with its antiangiogenic function partially dependent on one of its compounds, gallic acid [13,51]. The other RS compounds, rutin, myricetin, and hyperoside, have also exhibited antiangiogenic activities against cancer [52–54]. Therefore, it would be worthwhile to examine the antiangiogenic activity of RS extract in a laser-induced neovascularization wet AMD mouse model [9].

5. Conclusions

In conclusion, RS extract suppressed H_2O_2 and HFD-caused oxidative stress and inflammation in human RPE cells and mouse retina. RS extract also suppressed bodyweight gain and lowered cholesterol and triglyceride levels in the serum of mice fed with HFD. The data suggest that RS extract may offer therapeutic potential for preventing the development and progression of AMD.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions

Investigation, ML, SW, YW, JZ, JC, XP, QY and ZZ; conceptualization, ZZ and ZT; writing - original drafting and revising, ML and ZZ. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The animal work of this study was approved by the Animal Ethics and Welfare Committee, Hunan University of Chinese Medicine (SYXK (Xiang) 2019-0009, approved date 10 January 2019).

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/j.fbl2811279.

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