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Food Science and Human Wellness

journal homepage: <https://www.sciopen.com/journal/2097-0765>

Improvement of ellagic acid from *Rosa roxburghii* Tratt on MASLD via PPAR γ signaling pathway

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ABSTRACT: Metabolic dysfunction-associated steatotic liver disease (MASLD) poses a global health threat, necessitating natural therapeutics. *Rosa roxburghii* Tratt (RRT), a medicinal and edible resource, shows anti-MASLD potential but lacks defined bioactive components and mechanisms. Using UHPLC-HRMS analysis and network pharmacology, we identified ellagic acid (EA) as RRT's primary bioactive component against MASLD. In high-fat diet (HFD)-fed mice and oleic acid (OA)-induced hepatocytes, EA alleviated hepatic steatosis, reduced serum and liver triglycerides (TG) and total cholesterol (TC), and lipid droplet-associated marker *Plin2* expression. Mechanistically, EA inhibited PPAR γ transcription and translation, and concurrently downregulated the mRNA expression of lipid metabolism-related genes associated with the PPAR γ signaling pathway (*Cebpa*, *Acc1*, *Fasn*, *Cd36*, *Hmgcr*, *Srebf2*) in MASLD mice. Similar regulatory effects were also observed in two hepatic cell-based MASLD models (*Acc1*, *Hmgcr* and *Srebf2*). Pharmacological studies with PPAR γ agonist (pioglitazone) and antagonist (GW9662) confirmed EA counteracts lipid dysregulation via PPAR γ -dependency. This work establishes EA as a novel PPAR γ -targeting phytochemical restoring hepatic lipid homeostasis, providing a molecular foundation for plant-derived MASLD interventions and highlighting RRT's translational promise.

Keywords: Metabolic dysfunction-associated steatotic liver disease, *Rosa roxburghii* Tratt, ellagic acid, PPAR γ signaling pathway

1. Introduction

Metabolic dysfunction-associated steatotic liver disease (MASLD) represents a global health crisis affecting approximately 25% of the population^[1]. It is characterized by excessive hepatic lipid accumulation and metabolic dysregulation, frequently progressing to Metabolic-Associated Steatohepatitis (MASH), cirrhosis, and hepatocellular carcinoma^[2]. Despite its escalating prevalence, no pharmacotherapies are currently approved specifically for MASLD. The pathogenesis of MASLD is multifactorial, involving dysregulated lipid metabolism, oxidative stress, inflammation, and insulin resistance, which collectively pose significant challenges to drug development^[3]. Consequently, the identification of safe and effective therapeutic strategies is imperative.

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Received 8 July 2025
Received in revised form 30 October 2025
Accepted 14 December 2025

Medicinal and edible plants are highly valued for their dual roles as dietary resources and therapeutic agents. They are rich in bioactive compounds, which can regulate multiple targets in the body and have a favorable safety profile. Because of this, they are considered premium sources for developing new drugs^[4,5]. Certain medicinal and edible plants can effectively regulate metabolic disorders, making them promising for managing MASLD^[6,7]. *Rosa roxburghii* Tratt (RRT), a shrub in the Rosaceae family, is widely distributed in Southwest China, particularly in Guizhou, Yunnan, and Sichuan^[8]. Studies indicate that RRT is a typical medicinal and edible plant. Its fruit can be consumed fresh or processed into various products, such as juice, jam, and wine. It is also rich in nutrients and bioactive compounds, giving it significant medicinal value. As a folk medicinal material listed in "Guizhou Province Traditional Chinese Medicine and National Medicine Quality Standard" (2003 edition), RRT has demonstrated multiple pharmacological effects, including anticancer^[9], antioxidant^[10], anti-atherosclerotic^[11], hypoglycemic^[12], and anti-inflammatory activities^[13]. Previous studies have confirmed that RRT significantly ameliorates hepatic injury in mice with high-fat diet (HFD)-induced MASLD^[14,15]. However, the specific bioactive constituents responsible for these effects and their underlying mechanisms of action remain elusive.

Ellagic acid (EA), a natural polyphenol compound also known as hexahydroxydiphenic acid, is ubiquitously distributed in pome fruits, nuts, and plant tissues^[16,17], and is also present as a major compound in RRT^[18]. It exerts multifaceted pharmacological effects, including antioxidant, neuroprotective, hepatoprotective, antifibrotic, and anti-atherosclerotic activities, and has been demonstrated to ameliorate chronic diseases^[17,19-21]. Notably, recent studies indicate that EA improves dyslipidemia in diabetic models through modulation of lipid metabolism^[22]. In this study, we employed ultra-high-performance liquid chromatography coupled with high-resolution mass spectrometry (UHPLC-HRMS) to analyze the bioactive constituents in RRT. Combined with network pharmacology, our findings suggest that EA may be one of the key active compounds in RRT responsible for its anti-MASLD effects. However, whether the benefits of RRT against MASLD are attributable to EA, and whether EA ameliorates MASLD by regulating lipid metabolism to reduce hepatic steatosis, as well as the underlying molecular mechanisms, remain unclear. Our present study demonstrates that EA may serve as a novel PPAR γ -targeting phytochemical, capable of restoring hepatic lipid homeostasis through modulation of the PPAR γ signaling pathway. This discovery provides a molecular basis for developing plant-based therapeutics against MASLD and highlights the translational potential of RRT in metabolic liver disorders.

2. Materials and methods

2.1 Materials

RRT fruit juice was sourced from Guizhou Peihua Agricultural Science and Technology Development Co.,Ltd (Guizhou, China). EA standards was purchased from Weikeqi Biological Technology Co., Ltd (ChengDu, China). DMEM medium, MEM medium and fetal bovine serum (FBS) were purchased from Welgene (Daegu, Korea). Oil Red O kit and Masson's trichrome staining kit were purchased from Solarbio Technology Co., Ltd (Beijing, China). Triglyceride (TG) assay kit and total cholesterol (TC) assay kit were

purchased from Jiancheng Institute of Bioengineering (Nanjing, China). Trizol reagent was purchased from Invitrogen (Carlsbad, USA). SYBR Green Premix Pro Taq HS qPCR Kit was purchased from AG (ChangSha, China). Antibodies against PPAR γ and GAPDH were purchased from Proteintech (Wuhan, China).

2.2 RRT extracts sample preparation

The RRT juice was evaporated and concentrated through rotating evaporation and vacuum freeze-drying. Finally, the RRT freeze-dried powder was stored at 4°C in sterilized glass bottles.

2.3 The bioactive components of RRT detection using UHPLC-HRMS and subsequent screening

Chemical profiling of RRT was conducted using UHPLC-HRMS. For the RRT freeze-dried powder samples, data acquisition was guided by the following identification criteria: mass error < 25 ppm for precursor ions, and MS/MS spectral match score > 0.7 against high-resolution spectral libraries. Compounds identified in both positive and negative ionization modes were consolidated, with duplicates excluded. All annotated constituents were systematically classified according to the NPCClassifier rule-based system to characterize the phytochemical composition of RRT freeze-dried powder.

The base peak chromatograms (BPC) of the positive and negative ion modes of the RRT freeze-dried powder samples were analyzed. Peaks with high abundance were confirmed for their peak shapes and their second-order spectra were examined. The chromatographic peaks were sequentially numbered. Subsequently, the compounds corresponding to these peaks were preliminarily screened. The Swiss ADME platform (<http://www.swissadme.ch/>) was used to screen the compounds based on gastrointestinal absorption (GI absorption) score of “high” and druglikeness passing two “yes” criteria. Meanwhile, the TCMSP database (<https://tcmsp-e.com/>) was employed to screen the compounds based on oral bioavailability (OB > 20) and druglikeness (DL > 0.18). Compounds that met the screening criteria in both databases were retained and ranked according to OB and DL, respectively. After comprehensive analysis, the most optimal bioactive constituent of RRT, referred to as EA, was identified.

2.4 Network pharmacology predicts potential targets and pathways for EA in the treatment of MASLD

The SMILES molecular structure of EA was retrieved from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>). This structure was then utilized to predict its potential targets using both Swiss TargetPrediction (www.swisstargetprediction.ch/) and Super-PRED (<https://prediction.charite.de/>). Candidate genes associated with MASLD were retrieved from the GeneCards (<https://www.genecards.org/>) and OMIM (<https://www.omim.org/>) databases. The gene lists from both databases were integrated and duplicates were removed to construct a comprehensive MASLD-related gene set. Subsequently, intersection analysis was performed between the predicted targets of EA and the MASLD gene set to identify potential therapeutic targets of EA in the treatment of MASLD. Finally, KEGG pathway enrichment analysis (FDR < 0.05) was conducted using the DAVID database to explore the underlying molecular mechanisms of these targets.

2.5 Establishment of the HFD-induced mouse MASLD model and treatment with EA

Male C57BL/6J mice (6 weeks old, 18–20g) were purchased from Chongqing Tengxin Biotechnology Co., Ltd. (Chongqing, China), and were placed in a specific pathogen-free (SPF) room maintained at an air-conditioned temperature (23–25 °C) and a relative humidity of 50–60% on a 12 hour light/dark cycle. All animal care procedures and experiments were approved by the Experimental Animal Ethics Committee of Guizhou University (Approval Number: EAE-GZU-2024-E047). These mice were randomly assigned to one of four groups (n=8 per group): the Normal Diet group (ND group), the High-Fat Diet group (HFD group), the HFD + low-dose EA-treated group (HFD + EA 10 mg/kg group), and the HFD + high-dose EA-treated group (HFD + EA 30 mg/kg group). The ND group received a normal diet (12.95% kcal from fat, 24.02% protein, 63.03% carbohydrates), while the HFD group received a high-fat diet (60.93% kcal from fat, 19.79% protein, 19.27% carbohydrates). To confirm successful establishment of the MASLD model, three mice were randomly selected from each of the ND and HFD groups at 14 weeks, weighed, euthanized, and their blood and liver tissues were collected for analysis. Beginning at week 15, mice in the HFD+EA-treated groups received daily oral gavage of EA at doses of either 10 mg/kg or 30 mg/kg (in 200 µl volume), determined based on preliminary experiments which indicated this dosage did not affect food intake or behavioral status. Concurrently, the ND and HFD groups received daily gavage of 200 µl sterile saline. Water was available ad libitum throughout the study. At the end of the 32-week experiment, all mice were weighed and orbital blood was collected prior to euthanasia. Liver and other organs (heart, spleen, lung, kidney) were then harvested and weighed individually. The liver index was calculated as (liver weight/body weight) × 100. The experimental procedure is outlined in Fig. 1 (created using Figdraw).

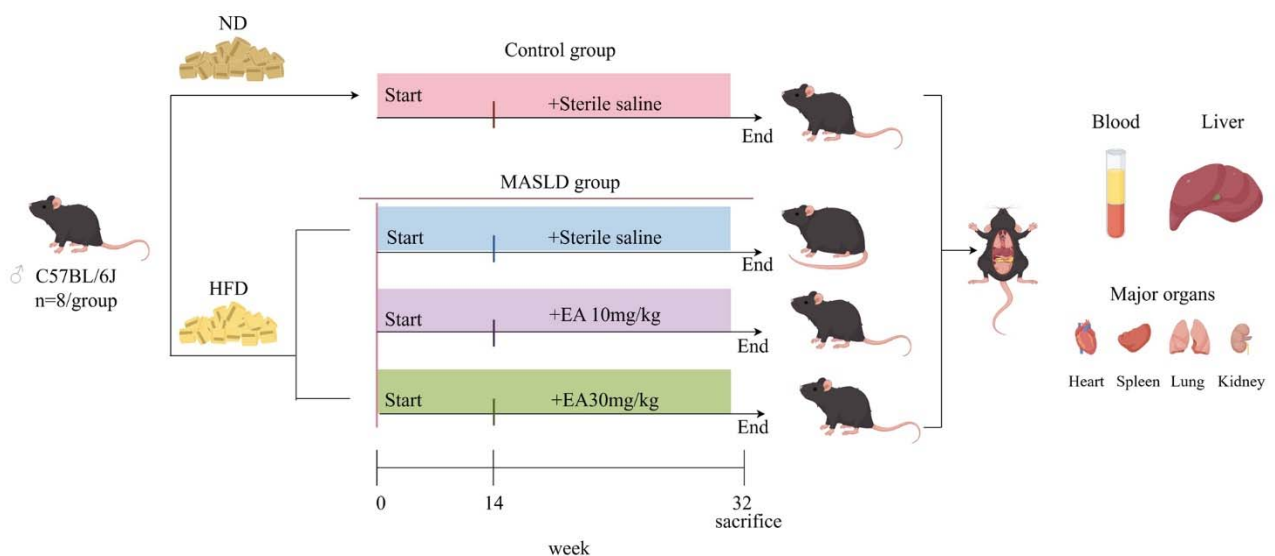


Fig 1. Schematic diagram of the experimental procedure in mice. n = 8 per group.

2.6 Establishment of OA-induced hepatocyte cell MASLD model and treatment with EA, PPAR γ agonist or antagonist

The murine hepatic AML12 cell line and human hepatoma HepG2 cell line were obtained from the Cell Bank of the Chinese Academy of Sciences (Shanghai Institute of Life Sciences, China). AML12 cells and

HepG2 cells were cultured in DMEM and MEM, respectively. Cells were cultured within a humidified atmosphere with 5% CO₂ at 37 °C.

The cells were divided into four groups: the Control group (Con group), the oleic acid (OA)-treated group (OA group), the OA + low-dose EA-treated group (OA + EA 50 µM group), and the OA + high-dose EA-treated group (OA + EA 100 µM group). Based on preliminary experiments, a MASLD cell model was established by treating AML12 or HepG2 cells with 0.2 mM OA for 24 hours. In the OA+EA groups, cells were co-treated with 0.2 mM OA and either 50 µM or 100 µM EA. In addition, we have also established PPAR γ agonist or antagonist combined treatment groups, 30 µM pioglitazone (PPAR γ agonist) or 10 µM GW9662 (PPAR γ antagonist) was added along with 0.2 mM OA and 100 µM EA. All treatments were maintained for 24 hours, after which cells were collected for further analysis.

2.7 Histopathological examination

The liver and other vital organs, including the heart, spleen, kidneys, and lungs, were fixed in 10% neutral-buffered formalin for 24 hr, processed through a graded ethanol series and xylene, then paraffin-embedded. Sections (4 µm) were stained with hematoxylin and eosin (H&E) for histopathological assessment. The representative fields of H&E-stained liver sections were subsequently evaluated using the NAFLD activity score (NAS) system, which quantifies four aspects: steatosis, lobular inflammation, hepatocellular ballooning, and fibrosis^[23].

2.8 ALT and AST measurements

The important indicators of liver function, serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were quantified using standardized assays. Following orbital blood collection at termination, samples were allowed to clot at room temperature and then centrifuged (e.g., 3,000 × g for 10-15 min at 4 °C) to isolate serum. The enzymatic activities of ALT and AST in the resultant serum were measured spectrophotometrically using commercially available reagent kits on an automated clinical chemistry analyzer, following the manufacturers' protocols. Enzyme activities are expressed in units per liter (U/L).

2.9 TG and TC measurements

The lipid content, including TG and TC, in hepatocyte cell, blood, and liver tissue of mice in per group was assessed using kits according to the manufacturer's instructions.

2.10 Lipid droplets content detection

The lipid droplets content of hepatocyte cell and mice liver tissues were detection by Oil Red O staining or Bodipy493/503 immunofluorescent staining. Oil Red O staining was performed according to the manufacture's instructions. Briefly, cells or frozen sections were washed twice with phosphate buffered saline (PBS), fixed with 4% paraformaldehyde (PFA) at room temperature for 2 h, rinsed twice with distilled water, and incubated with 60% isopropanol for 20-30 s. Freshly prepared Oil Red O solution was then added for 10-20 min in the dark. After differentiation with 60% isopropanol and washing with distilled water, sections were mounted with Neutral Balsam Mounting Medium and imaged under a light microscope. Under a 100×

magnification field of view, 3 non-overlapping fields of view were randomly selected in the liver of each mouse. The lipid droplet area was quantified using ImageJ software and used as a quantitative indicator. The Bodipy 493/503 staining was also conducted following the manufacturer's protocol. Cells cultured on 14 mm glass coverslips were fixed with 4% PFA for 10 min at room temperature after PBS washing. Following three PBS washes, cells were incubated with 100 μ L of 10 μ M Bodipy 493/503 for 30 min at room temperature. After another PBS wash, samples were mounted with an anti-fade reagent containing DAPI. Fluorescence images were captured using an Olympus SpinSR10 microscope. The fluorescence intensity of cells was measured using ImageJ software.

2.11 Masson's trichrome staining

Masson's trichrome staining was conducted following the manufacturer's guidelines. In brief, the tissue sections were immersed in Weigert's working hematoxylin for 10 minutes. After rinsing with distilled water, the sections were stained with Masson's trichrome solution for an additional 10 minutes. The sections were then rinsed in 95% alcohol, dehydrated, cleared, and covered with a coverslip. The relative area of collagen fibers was quantitatively analyzed as follows: under a magnification of 100 $\times$, three non-overlapping fields of view were randomly chosen from the liver of each mouse. The area of the blue-stained regions was measured using ImageJ software to determine the relative area of collagen fibers. The average values obtained served as quantitative indicators of collagen fibers in the liver of each mouse.

2.12 Real-time quantitative PCR (RT-qPCR)

The mRNA levels of lipid metabolism genes in hepatocyte cell or mice liver tissue were detected using RT-qPCR, respectively. Total RNA was extracted from tissues using Trizol reagent (Invitrogen, Carlsbad, USA). First-strand cDNA was synthesized from purified RNA. RT-qPCR was performed on a CFX96 system (Bio-Rad, CA). RT-qPCR was performed by using SYBR Green Premix Pro Taq HS qPCR Kit. Specific primers were designed using NCBI BLAST, as detailed in Table S1. The relative mRNA levels were calculated by $2^{-\Delta\Delta C_t}$.

2.13 Western blotting(WB)

The protein expression of PPAR γ in the mice liver tissue was detected by WB. Total protein was extracted, and proteins were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and then transferred onto polyvinylidene difluoride (PVDF) membranes. After blocking with 5% nonfat dry milk, the PVDF membranes were incubated with anti-PPAR γ antibody (1:5000 dilution) and anti-GAPDH antibody (1:1500 dilution) overnight at 4 $^{\circ}$ C, respectively. After washing, membranes were incubated with secondary antibodies for 2 hours at room temperature. Protein signals were detected using an ECL chemiluminescent substrate. The intensity of the signal on the film was quantitated using a computer-assisted imaging system and normalized to the corresponding signal for GAPDH.

2.14 Immunofluorescence (IF) staining

The protein expression of PPAR γ in HepG2 cells was detected by IF staining. Briefly, cells were fixed with 4% PFA (25 °C, 20 min), blocked with PBS containing 5% goat serum and 0.1% Triton X-100 (30 min). Cells were incubated with anti-PPAR γ antibody (1:500 dilution) for 2 hour at room temperature. After PBS/0.1% Tween-20 washes, cells were incubated with secondary antibodies (goat anti-mouse 488, 1:2000 dilution) for 30 minutes. The slides were mounted with ProLongTM Gold antifade Reagent with DAPI (Invitrogen, Carlsbad, USA). Fluorescence images were captured using an Olympus SpinSR10 microscope. The fluorescence intensity of cells was measured using ImageJ software.

2.15 Statistical analysis

Data from the groups are expressed as the means $\pm$ standard deviations (SD). Means were compared by a one-way analysis of variance (ANOVA) and t-test using SPSS software version 20.0 (IBM, Armonk, NY, USA) to determine if there were statistically significant differences. A P -value < 0.05 indicated a statistically significant difference.

3. Results

3.1 Screening identifies EA as a candidate for MASLD therap

To systematically identify bioactive constituents of RRT and evaluate their potential role in MASLD, we conducted UHPLC-HRMS profiling to analyze the bioactive components in the RRT freeze-dried powder. A total of 1,693 compounds were identified in positive ion mode and 614 compounds in negative ion mode, resulting in 2,256 unique compounds after removing duplicates. Alkaloids were the most abundant class, accounting for approximately 23%, followed by terpenoids, shikimates and Phenylpropanoids and fatty acids (Fig. 2A). BPC analysis highlighted 28 prominent chromatographic peaks (Fig. 2B), corresponding to high-abundance compounds (Table S2). Among these, terpenoids were the most represented group, followed by shikimates and phenylpropanoids, alkaloids, carbohydrates, and fatty acids (Fig. 2C). Preliminary screening based on OB > 20 and DL > 0.18 criteria was conducted, and candidate compounds (Epicatechin, Ellagic acid, and Arjunic acid) were ranked accordingly (Fig. 2D). EA emerged as the optimal compound according to combined OB and DL scores, suggesting its potential as a key active component.

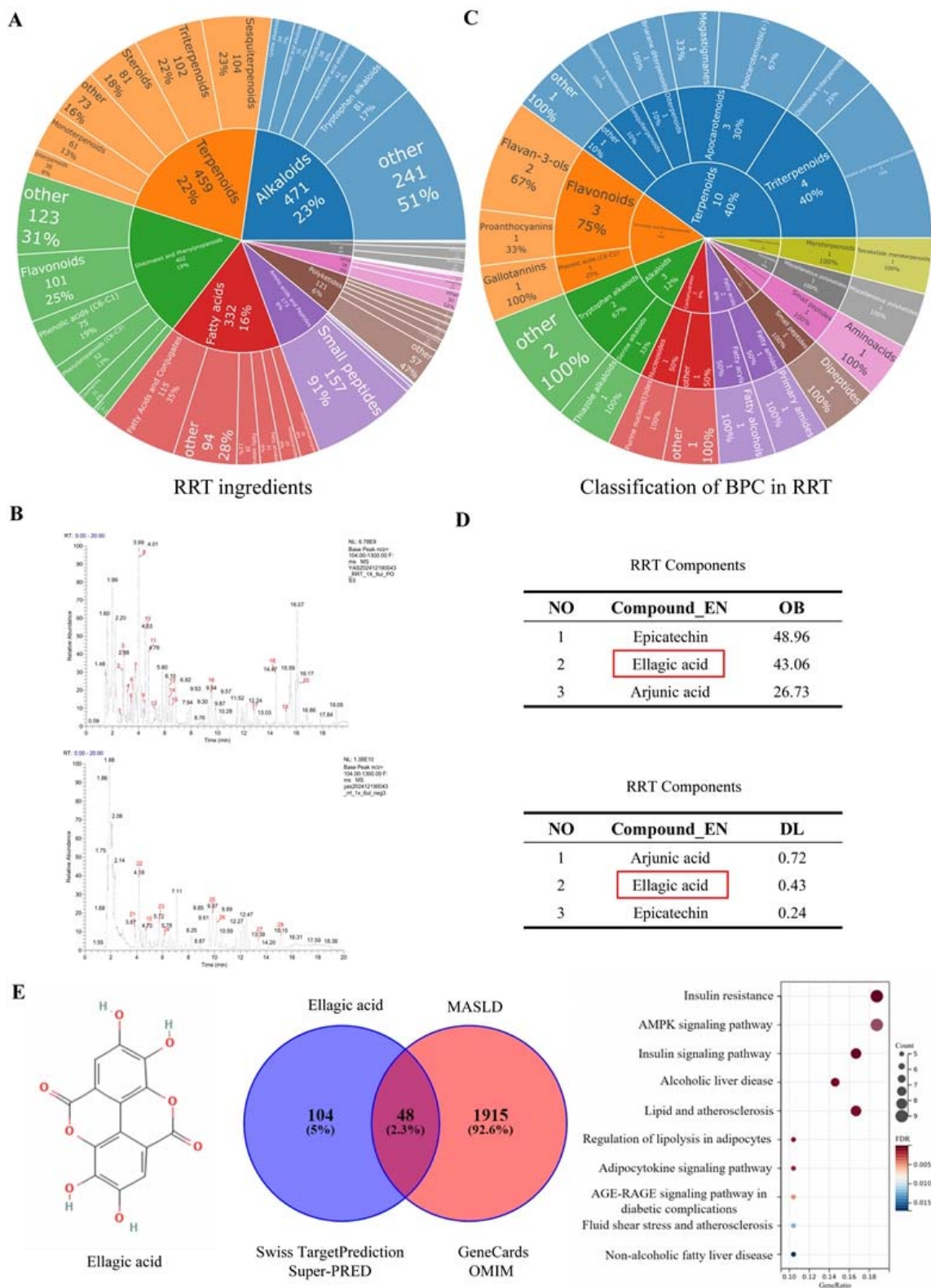


Fig 2. Screening Bioactive Compounds from RRT and Identifying EA as a Candidate for MASLD Therapy. (A) Proportional distribution of identified compounds in RRT across different chemical classes. (B) BPC of RRT in positive and negative ion modes. (C) Distribution of high-abundance compounds among chemical classes in the BPC. (D) Ranking of candidate compounds based on OB and DL. (E) Molecular formula of EA, Venn diagram showing the intersection between predicted targets of EA and MASLD-associated targets, and KEGG pathway enrichment analysis of 48 shared targets.

To investigate the therapeutic potential of EA in MASLD and identify its possible molecular targets, we predicted its putative target proteins using public databases and intersected them with known MASLD-related targets, yielding 48 common targets (Table S3). KEGG pathway enrichment analysis revealed that these

targets were significantly enriched in lipid metabolism-related pathways, including insulin signaling, AMPK signaling, alcoholic and non-alcoholic fatty liver disease, adipocytokine signaling, and atherosclerosis (FDR < 0.05) (Fig. 2E)(Table S4). These findings suggest that EA may exert Attenuates MASLD effects through modulation of lipid metabolic pathways. Taken together, our data highlight EA as a promising bioactive compound from RRT, providing a mechanistic basis for its potential application in the prevention and treatment of MASLD.

3.2 EA alleviates hepatic cell lipid accumulation in the HFD-induced mouse MASLD model

To further investigate the role of EA in MASLD, we established an HFD-induced murine MASLD model and subsequently administered EA to assess its effects on hepatic tissue. Compared to the ND group, mice fed an HFD for 14 weeks exhibited significantly increased body weight ($P < 0.001$) and pronounced hepatocyte steatosis, confirming successful establishment of the MASLD model (Fig. 3A). Following 18 weeks of EA treatment (concluding at experimental week 32), HFD + EA-treated mice demonstrated significantly reduced body weight, liver index, serum ALT and AST levels relative to the HFD group ($P < 0.05$, $P < 0.01$, $P < 0.001$)(Fig. 3B-C). Histopathological analysis combined with Oil Red O staining revealed that EA significantly attenuated hepatic steatosis and lipid droplet accumulation. Given that fibrosis progresses alongside with the development of MASLD, we also utilized Masson staining to assess hepatic fibrotic changes. The histopathological changes of the liver, when combined with the results of Masson staining, suggest that the mouse model established in this experiment has not yet exhibited significant pathological changes associated with liver fibrosis. Moreover, EA also decreased the mRNA expression level of the lipid droplet-associated marker *Plin2* in HFD-fed mice ($P < 0.001$)(Fig. 3D), reduced both serum and hepatic TG and TC contents ($P < 0.01$, $P < 0.001$)(Fig. 3E). These effects were more pronounced in the high-dose EA group (30 mg/kg) compared to the low-dose group (10 mg/kg). Additionally, histopathological examination of major organs (heart, spleen, lung, kidney) showed no significant morphological alterations, except for varying degrees of lipid accumulation in renal tubular epithelial cells observed in both the HFD and HFD+EA treatment groups (Fig. S1). Collectively, these findings indicate that EA treatment effectively ameliorates HFD-induced hepatic lipid accumulation in MASLD mice without apparent systemic toxicity.

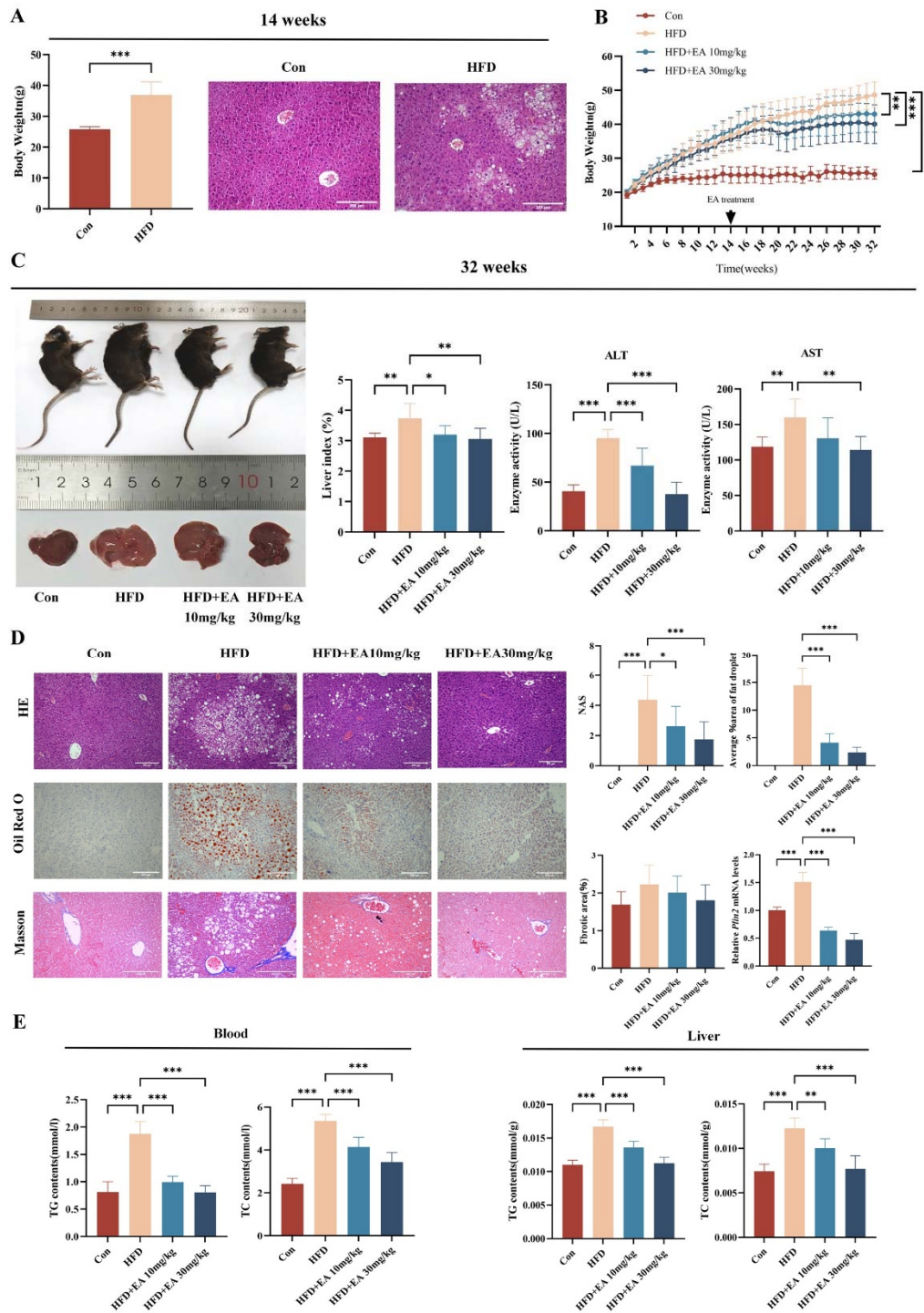


Fig 3. EA alleviates hepatic Lipid Accumulation in the HFD-induced mouse MASLD model. (A) Body weight and hepatic histological alterations in mice following 14 weeks of dietary intervention. (B) Body weight changes over 32 weeks of mice in each group. (C) Representative images of mouse morphology and liver, as well as liver index, and ALT and AST levels at week 32. (D) Representative images of H&E (H&E $\times 100$; Scale bar=200 μ m), Oil Red O (Oil Red O $\times 100$; Scale bar=200 μ m), and Masson's trichrome staining (Masson $\times 200$; Scale bar=200 μ m) of the liver, along with the NAS and relative mRNA expression levels of *Plin2*. (E) Serum and hepatic TG and TC contents. $n = 8$ per group. Data are presented as mean $\pm$ SD. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

3.3 EA mitigates OA-induced lipid accumulation in hepatocytes in vitro

To further assess whether EA could alleviate OA-induced hepatic lipid accumulation, we established in vitro models of OA-induced MASLD using AML12 and HepG2 cells. The results indicated that, in comparison to the OA group, EA treatment markedly decreased lipid droplet content, the mRNA expression

levels of *Plin2* (*PLIN2*), and the levels of TG and TC in both AML12 and HepG2 cells, in a concentration-dependent manner ($P < 0.05$, $P < 0.01$, $P < 0.001$)(Fig. 4). These findings also confirm that EA effectively ameliorates OA-induced hepatic lipid accumulation at the cellular level.

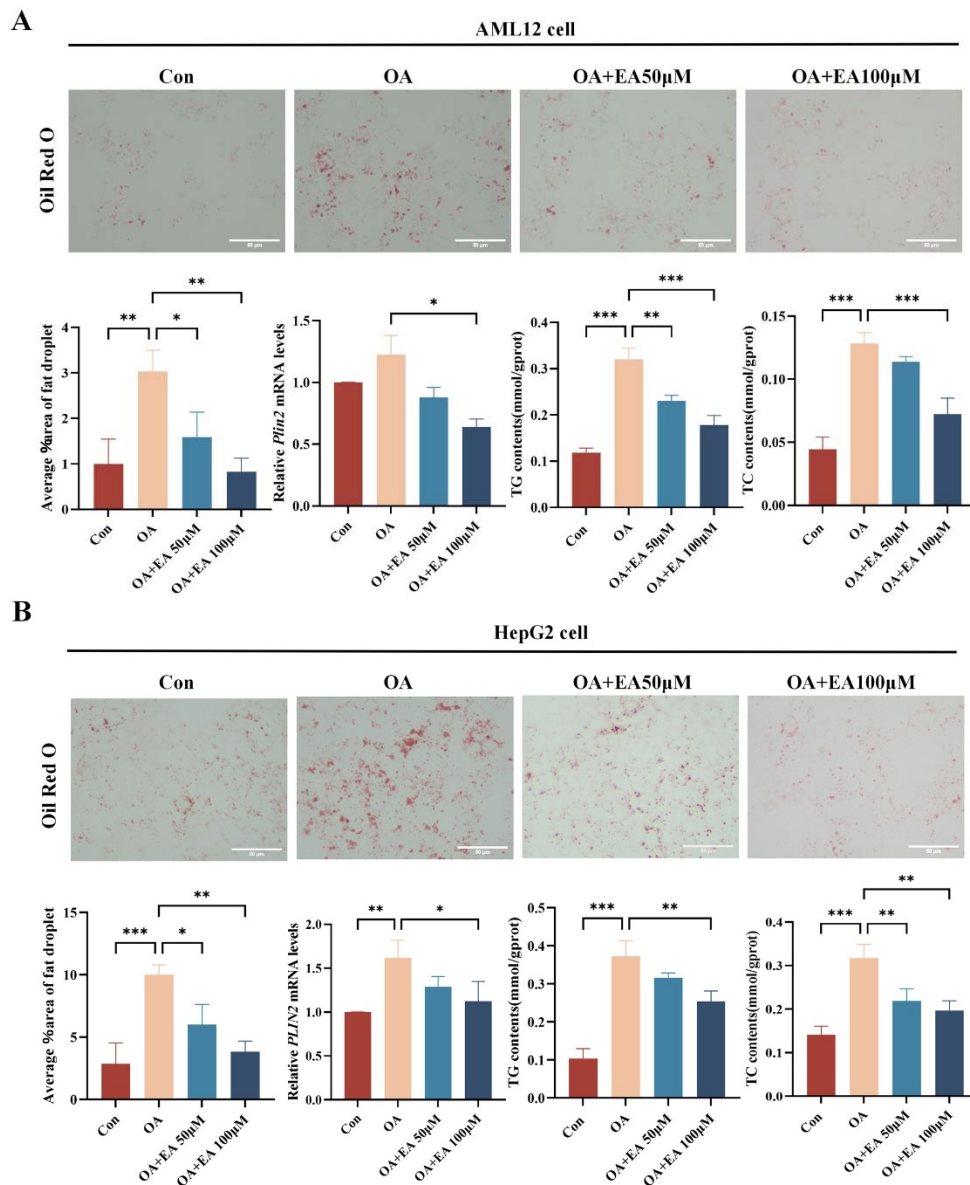


Fig 4. EA mitigates OA-induced lipid accumulation in hepatocytes in vitro. (A) Oil Red O staining, TG and TC contents, and relative mRNA expression levels of *Plin2* in AML12 cells of each group. (B) Oil Red O staining, TG and TC contents, and relative mRNA expression levels of *PLIN2* in HepG2 cells of each group. Data are presented as the mean $\pm$ SD. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

3.4 EA alleviates *PPAR γ* and downstream target genes of the *PPAR γ* signaling pathway upregulation in HFD and OA-induced MASLD models

As presented in Section 3.1, KEGG pathway analysis of EA's predicted targets showed significant enrichment for genes involved in lipid metabolism. The presence of both direct and indirect associations suggests that EA likely ameliorates disease by regulating lipid metabolic processes. Hepatic lipid accumulation, which is primarily driven by dysregulated lipid metabolism, represents the initial pathological step in the development of MASLD. *PPAR γ* , widely recognized as a master regulator of lipid metabolism, plays a central role in biological processes such as lipogenesis, adipocyte differentiation, and lipid

homeostasis. To investigate whether EA modulates lipid metabolism via the PPAR γ signaling pathway, we first examined the mRNA and protein expression levels of PPAR γ in liver tissues from HFD-fed mice and in two hepatocyte models treated with OA. The results indicated that HFD feeding or OA treatment led to a significant upregulation of PPAR γ expression at both the mRNA and protein levels. Conversely, EA administration effectively attenuated this upregulation ($P < 0.01$, $P < 0.001$)(Fig. 5 A-C). Additionally, IF staining of PPAR γ in HepG2 cells also demonstrated that OA treatment not only significantly enhanced PPAR γ protein fluorescence intensity but also induced the translocation of PPAR γ into the cell nucleus, while EA treatment effectively reduced both its protein expression and nuclear translocation ($P < 0.05$)(Fig. 5C). Furthermore, we examined the mRNA expression levels of several downstream target genes of the PPAR γ pathway. The results indicated that EA decreased the mRNA expression of key genes involved in lipid metabolism, such as those responsible for fatty acid binding, transport, and synthesis (*Cd36*, *Acc1*, *Fasn*, *Hmgcr*, and *Srebf2*), compared to mice with HFD. Additionally, the lipid metabolism-related gene *Cebpa* was also downregulated, which cooperates with PPAR γ to regulate adipocyte differentiation and lipid synthesis ($P < 0.01$, $P < 0.001$)(Fig. 6A). In vitro experiments have also confirmed that treatment with EA significantly reduced the expression of lipid synthesis genes, including *Acc1*(*ACCI*), *Hmgcr*(*HMGCR*), and *Srebf2*(*SREBF2*), in both AML12 and HepG2 cells compared to cells with OA-treated ($P < 0.01$, $P < 0.001$) (Fig. 6B). This indicates that EA can regulate the expression of lipid-metabolism-related genes and participate in hepatic lipid-metabolism regulation by inhibiting PPAR γ signaling pathway in MASLD models.

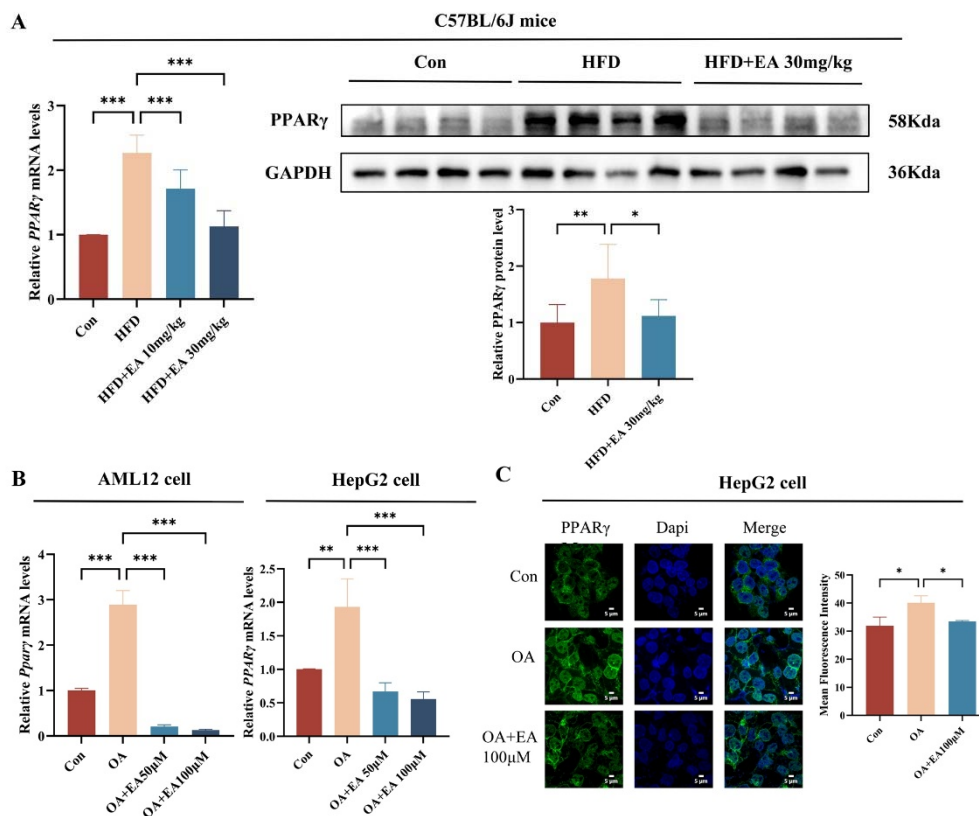


Fig 5. EA alleviates PPAR γ upregulation in HFD and OA-induced MASLD models. (A) Relative mRNA and protein expression levels of PPAR γ in liver tissue of mice in each group. $n = 8$ per group. (B) Relative mRNA expression levels of *Pparγ* (PPAR γ) in AML12 cells and HepG2 cells of each group. (C) The PPAR γ protein expression in HepG2 cells of each group by IF staining. Data are presented as the mean $\pm$ SD. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

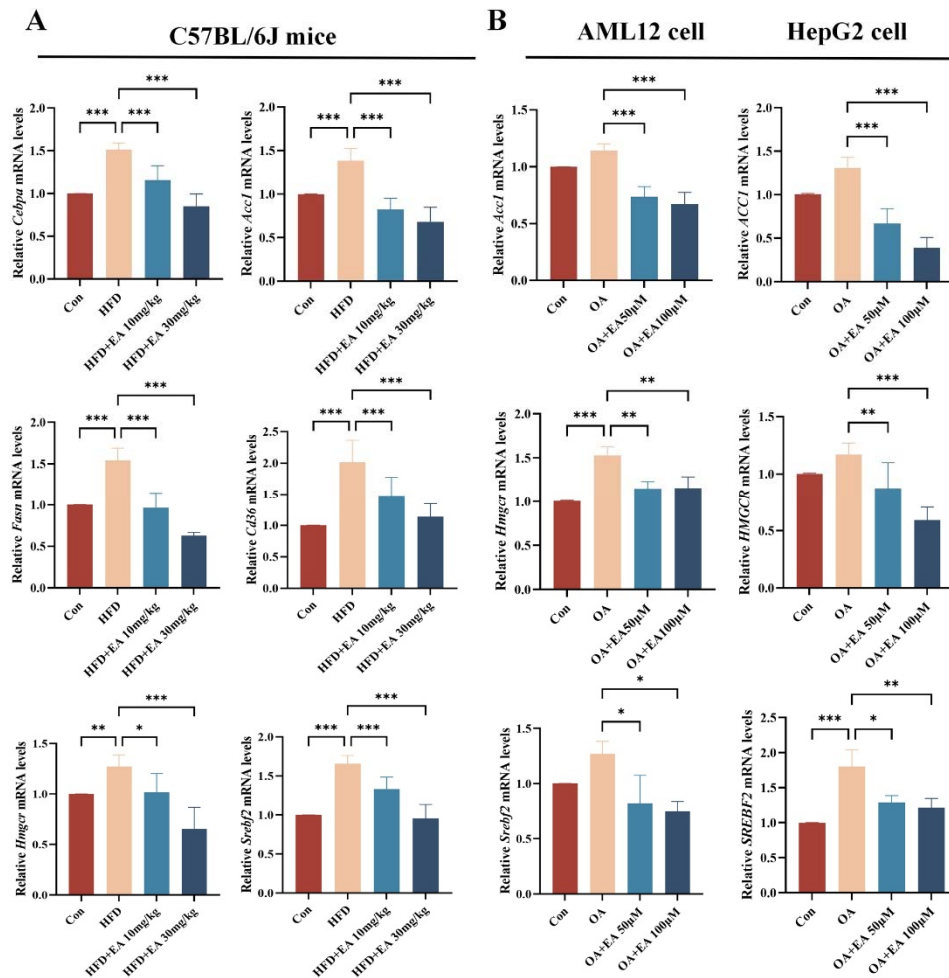


Fig 6. EA alleviates the upregulation of lipid - metabolism - related genes in HFD and OA - induced MASLD models. (A) Relative mRNA expression levels of lipid metabolism-related genes (*Cebpa*, *Acc1*, *Fasn*, *Cd36*, *Hmgcr* and *Srebf2*) in liver tissue of mice in each group. (B) Relative mRNA expression levels of lipid metabolism-related genes, including *Acc1* (*ACCI*), *Hmgcr* (*HMGCR*), and *Srebf2* (*SREBF2*), in AML12 and HepG2 cells across of each group. Data are presented as the mean $\pm$ SD. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

3.5. EA suppresses hepatic lipid accumulation by inhibiting the PPAR γ signaling pathway

To further investigate whether the effects of EA on lipid metabolism are mediated via PPAR γ signaling, we treated AML12 cells and HepG2 cells with either the PPAR γ agonist pioglitazone or the PPAR γ antagonist GW9662, respectively. The Bodipy 493/503 fluorescence staining results showed that, compared to EA treatment alone, co-treatment with pioglitazone exhibited a trend toward increased intracellular lipid droplet accumulation, although this was not statistically significant. Conversely, co-treatment with GW9662 significantly reduced intracellular lipid droplet accumulation in both AML12 and HepG2 cells ($P < 0.05$, $P < 0.01$, $P < 0.001$)(Fig. 7A). Consistently, pioglitazone reversed the EA-induced reduction in TG and TC contents, while GW9662 further enhanced their suppression ($P < 0.05$, $P < 0.01$, $P < 0.001$)(Fig. 7B). Furthermore, we performed RT-qPCR to assess the mRNA expression levels of *Cebpa* (*CEBPA*), *Cd36*(*CD36*) and *Fasn*(*FASN*). Compared to EA treatment alone, pioglitazone upregulated the mRNA expression of these genes, whereas GW9662 further suppressed their expression ($P < 0.05$, $P < 0.01$, $P < 0.001$), although some indicators did not reach statistical significance, trends were still observable (Fig. 7C).

Collectively, these findings suggest that EA may exert its protective effects against OA-induced hepatic lipid accumulation and MASLD progression by downregulating PPAR γ signaling pathway.

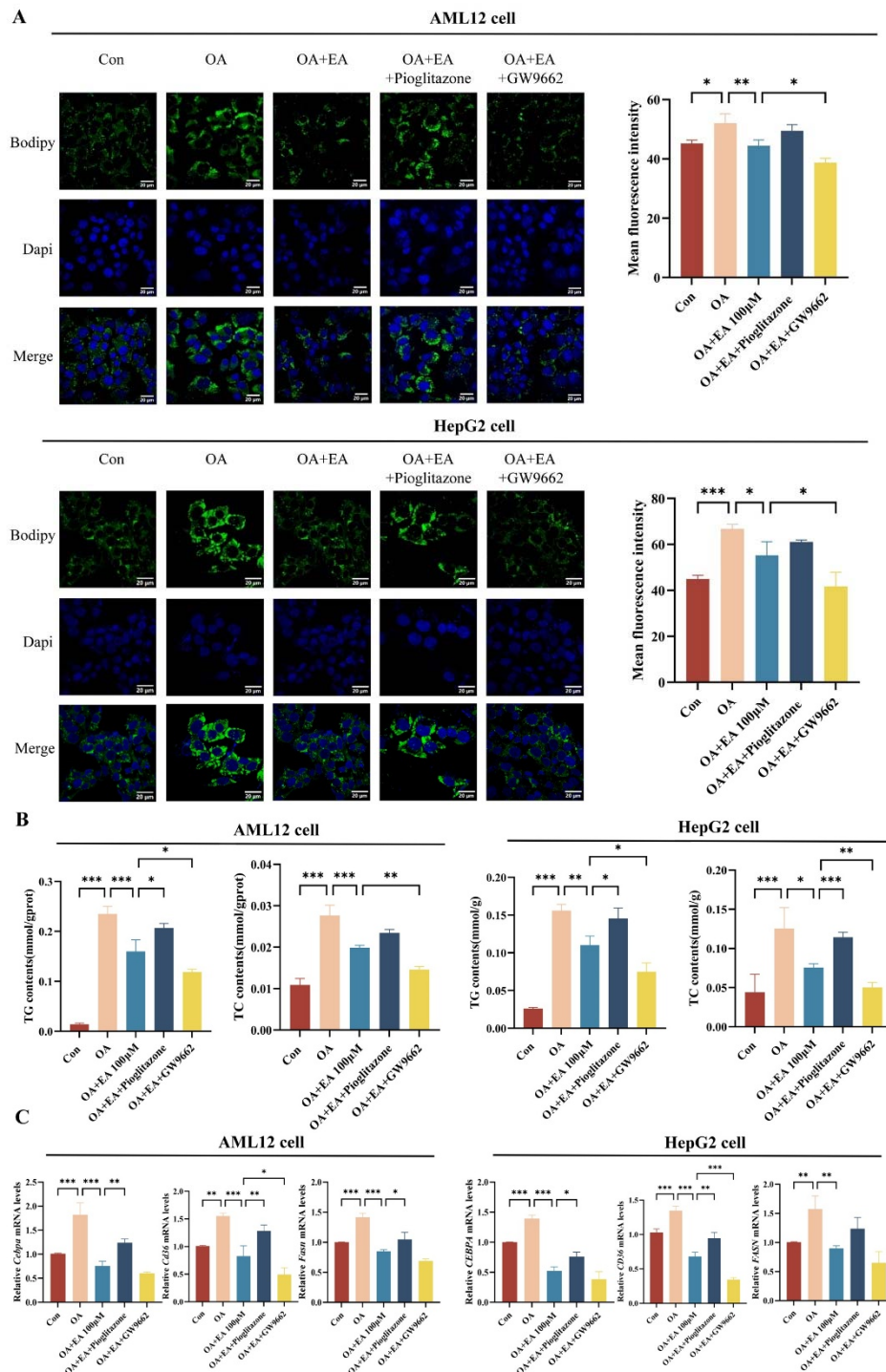


Fig 7. EA suppresses hepatic lipid accumulation through PPAR γ inhibition. (A) Lipid droplets contents in AML12 cells and HepG2 cells of each group by Bodipy 493/503 fluorescent dye staining. (B) TG and TC contents in AML12 cells and HepG2 cells of each group; (C) The relative mRNA expression of *Cebpa* (*CEBPA*), *Cd36* (*CD36*), and *Fasn* (*FASN*) in AML12 and HepG2 cells of each group. Data are presented as the mean $\pm$ SD. * P < 0.05, ** P < 0.01, *** P < 0.001.

4. Discussion

MASLD represents a growing global health burden linked to obesity and metabolic syndrome. Despite its high prevalence, therapeutic options for MASLD remain limited. Bioactive compounds derived from dietary

plants offer promising avenues for intervention^[6,24]. This study investigated the therapeutic potential of EA, a bioactive polyphenol derived from RRT, in MASLD. While EA is recognized for its anti-inflammatory properties, our findings demonstrate that it significantly alleviates hepatic lipid accumulation and reduces serum and hepatic TG and TC contents in both HFD-induced mice and OA-treated hepatocytes. Mechanistically, EA modulated lipid metabolism by inhibiting the PPAR γ signaling pathway. These results suggest EA as a promising bioactive agent for preventing and treating MASLD, offering a novel therapeutic strategy to address the currently limited therapeutic options for this condition.

RRT is a perennial deciduous shrub belonging to the genus *Rosa* in the family Rosaceae, and has a long history of use as a functional food. Its fruit contains various bioactive components, including polysaccharides, flavonoids, superoxide dismutase (SOD), and vitamin C, contributing to its diverse pharmacological effects^[25,26]. In recent years, RRT has drawn increasing attention as a medicinal and edible plant for its potential role in metabolic diseases, such as obesity^[27], diabetes^[28], hepatocellular carcinoma^[9]. Preliminary studies from our research group found that RRT extract significantly ameliorated hepatic lipid accumulation induced by HFD in mice, reduced serum TG and TC contents. However, its core active ingredients and underlying mechanisms remained unclear. In this study, using UHPLC-HRMS combined with network pharmacology analysis, EA was identified for the first time as a key component responsible for RRT's intervention against MASLD, and its molecular mechanism in regulating lipid metabolism was elucidated. These findings not only decipher the molecular basis of RRT's traditional medicinal properties but also provide a theoretical foundation for developing natural inhibitor-based targeted therapies for MASLD, highlighting the multi-target regulatory advantages of medicinal and edible plants in combating metabolic disorders.

EA, a naturally occurring polyphenol abundant in pomegranates, strawberries, nuts, and RRT, exhibits potent antioxidant, anti-inflammatory and antiproliferative properties, highlighting its therapeutic potential for metabolic and hepatic disorders. Studies have indicated that EA demonstrates multifaceted hepatoprotective effects: it attenuates liver fibrosis by inducing ferroportin-dependent ferroptosis through SNARE complex impairment^[29], counteracts ccl4-induced hepatotoxicity via Nrf2 pathway activation^[30] and ameliorates alcohol-related liver disease by regulating the gut microbiota–NPAS2–hepatic circadian axis^[31]. However, the therapeutic potential and mechanistic basis of EA in MASLD remain unelucidated. To investigate the therapeutic effects of EA on MASLD, we established MASLD models using HFD-fed mice and OA-treated cells to assess whether EA could alleviate hepatocyte lipid accumulation induced by HFD and OA. Notably, EA reduced lipid droplet formation and lowered TG and TC contents, mirroring its lipid-lowering effects.

While prior studies emphasized EA's properties, our work uniquely identifies PPAR γ as its primary molecular target, offering a mechanistic bridge between RRT's traditional use and modern metabolic therapeutics. PPAR γ , a member of the nuclear receptor superfamily, serves as a central regulator of lipid metabolism, adipocyte differentiation, and insulin sensitivity^[32,33]. The PPAR γ signaling pathway is a crucial cellular signaling mechanism that plays a significant role in lipid metabolism. It regulates adipocyte

differentiation, lipid synthesis, storage, and breakdown, as well as the secretion of adipokines, thereby contributing to the maintenance of systemic metabolic homeostasis. The overactivation of PPAR γ can lead to ectopic lipid deposition by regulating the expression of lipid-metabolism-related target genes downstream of the PPAR γ pathway, thereby accelerating the progression of MASLD and type 2 diabetes^[32]. Recent studies indicate that natural products have emerged as promising candidates due to their multi-target effects and low toxicity^[34]. For instance, Magnolol improved mitochondrial dysfunction involving PPAR γ pathway^[35]. In the present study, we identified that EA, a natural compound derived from RRT, may function as a PPAR γ antagonist. We found that EA inhibited HFD or OA induced PPAR γ expression at both the transcriptional and translational levels in mouse and cell based MASLD models. Additionally, our results indicated that in HepG2 cells, OA treatment significantly enhanced PPAR γ fluorescence intensity and promoted its nuclear translocation, whereas EA effectively reduced both PPAR γ expression and its translocation to the nucleus. Likewise, EA suppressed the mRNA expression of downstream target genes involved in the PPAR γ pathway, including those responsible for fatty acid binding, transport, and synthesis, thereby attenuating dysregulated lipid metabolism. This indicates that EA regulates hepatic lipid metabolism by inhibiting PPAR γ in MASLD models. We further treated the cells with a PPAR γ agonist and antagonist. In both cell models, compared to the EA treatment group, the PPAR γ agonist increased the expression of lipid-metabolism-related genes associated with PPAR γ , while the PPAR γ inhibitor had the opposite effect. This fully confirms our previous conclusions. Our findings reveal a novel PPAR γ modulation mechanism-partial suppression of pathological activation-and establish the first link between medicinal and edible plant bioactives and precision-targeted PPAR γ regulation. This provides an innovative paradigm for developing natural PPAR γ modulators that reduce lipid deposition without weight gain, laying a molecular foundation for MASLD dietary interventions.

This study elucidates the core mechanism by which EA, the principal bioactive component of RRT extract, ameliorates MASLD through targeted modulation of the PPAR γ signaling pathway. Experimental validation confirmed that EA effectively alleviates hepatic lipid metabolism disorders by suppressing PPAR γ -mediated lipid metabolic pathways and restoring cellular homeostasis. Notably, EA's role as a natural PPAR γ antagonist offers a novel perspective for deciphering the molecular mechanisms of medicinal and edible plants. From a translational medicine standpoint, this work not only elucidates the key molecular targets underlying EA's efficacy in ameliorating MASLD but also underscores the scientific value of RRT in the prevention and management of metabolic disorders. The established evidence chain-linking natural products, pathological amelioration, and defined molecular targets-provides an innovative framework for the development of plant-derived therapeutics, bridging traditional phytomedicine with modern precision pharmacology.

Although this study has preliminarily confirmed the therapeutic potential and mechanism of action of EA for MASLD, several limitations remain. First, the effective dose of EA in this mouse study, when converted to the human equivalent (0.81 - 2.43 mg/kg/day)^[36], is significantly lower than the reported human safe and effective dose of EA (100 - 200 mg/day) in the literature^[37]. This suggests that the dose of EA may be effective

and safe for humans. However, due to the differences in pharmacokinetics and pharmacodynamics among different species, further human trials are still needed to explore the human - equivalent dosage and safety margin to highlight the potential applicability of EA as a nutraceutical ingredient. Second, future studies should include receptor binding experiments to clarify whether EA acts as a direct ligand for PPAR γ . Third, given the relatively short treatment duration in our animal experiments and the limited scope of metabolite identification, further investigation is needed to explore the long-term effects and potential side effects of EA, as well as to provide a more comprehensive assessment of its bioavailability. Addressing these limitations in future studies will be essential for validating our findings and enhancing the understanding of EA's therapeutic potential. Moving forward, our research should focus on exploring the potential value of EA as a nutritional supplement. Specifically, future work should determine appropriate doses in humans, particularly among high-risk populations, while assessing its potential interactions and safety to promote its scientific development as a functional food ingredient.

Data availability

Data will be made available on request.

Acknowledgments

This research was supported by Guizhou Provincial Science and Technology Foundation (NO.ZK[2025]642, ZK[2024]Major030); Guiyang Science and Technology Foundation (NO.[2023]13-17) and Guizhou University Basic Research Program (NO. [2024]37); Guizhou University Introduces Talents Foundation (NO. [2022]51).

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