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Allicin-free garlic extract alleviates alcohol-induced liver injury in mice by reducing oxidative stress through activation of PPAR γ

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ABSTRACT: Garlic (*Allium sativum* L.) is well known for its antioxidant activity and has been demonstrated to protect against alcoholic liver disease (ALD); however, its pungent odor hinders its broader application. This study introduced allicin-free garlic extract (AFGE), and investigated its effect on alcohol-induced hepatotoxicity and the underlying mechanisms. ICR mice were administered AFGE at doses of 0.5, 1, and 2 g/kg for 30 days, followed by a single dose of alcohol (14 mL/kg) to simulate human binge drinking. AFGE significantly alleviated alcohol-induced hepatocellular injury, reduced hepatic lipid accumulation and oxidative damage. These antioxidant beneficial effects were further confirmed in aged mice, as evidenced by decreased hepatic levels of malondialdehyde and protein carbonyls, and increased levels of glutathione and catalase. Transcriptomic analysis and integrated bioinformatic analysis identified *Gpx3*, *Gsto2*, and *Gsta1* as core antioxidant genes involved in the protective effects against alcohol-induced hepatotoxicity. Further analysis with Cytoscape revealed that peroxisome proliferator-activated receptor gamma (PPAR γ) is a key transcription factor regulating the expression of these genes. Indeed, the hepatic abundance of PPAR γ was markedly upregulated by AFGE compared with the alcohol group, indicating the involvement of this transcription factor in the protective effects of AFGE. In addition, molecular docking and western blotting showed that alliin, one of the main components of AFGE, directly binds to and activates PPAR γ . Collectively, these findings demonstrate that AFGE mitigates alcohol-induced liver injury by activating PPAR γ -mediated transcription of antioxidant genes, supporting its potential as a functional food for the prevention and management of ALD.

Keywords: Alcoholic liver disease, Oxidative stress, Garlic extract, Peroxisome proliferator-activated receptor γ , Antioxidant activity

1. Introduction

Alcoholic liver disease (ALD) is among the most prevalent liver diseases worldwide, primarily caused by prolonged and excessive alcohol consumption. According to the World Health Organization^[1], excessive alcohol consumption caused nearly 3 million deaths and 115.9 million disability-adjusted life years (DALYs) globally in 2019, emerging as a serious public health challenge that particularly affects low- and middle-income countries. Without timely and effective intervention, ALD can progress from simple steatosis to alcoholic steatohepatitis, fibrosis, cirrhosis, and ultimately hepatocellular carcinoma^[2].

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The pathogenesis of ALD involves a complex interplay of multiple biological processes^[3] including alcohol metabolism that is affected by genetic and environmental factors, intrahepatic immune responses^[4], and intracellular events such as oxidative stress and lipid accumulation. Among these, alcohol-induced oxidative stress plays a critical role in promoting hepatic injury^[5]. Following absorption, alcohol is primarily metabolized via oxidative pathways catalyzed by alcohol dehydrogenase (ADH) and cytochrome P450 2E1 (CYP2E1), and to a lesser extent through non-oxidative pathways such as fatty acid ethyl ester synthesis^[2]. These processes generate reactive oxygen species (ROS), which contribute to cellular damage and ALD progression. In addition, alcohol impairs the cellular antioxidant defense system primarily through the depletion of glutathione (GSH), a key intracellular antioxidant^[6]. Consequently, ROS accumulate, resulting in sustained oxidative stress that is detrimental to liver cells. Extensive studies have demonstrated that activating and enhancing cellular defense systems to mitigate oxidative stress can effectively alleviate ALD, in part because critical regulators such as peroxisome proliferator-activated receptor gamma (PPAR γ), are suppressed by alcohol exposure^[7-8]. Currently, clinical management of ALD is largely limited to symptomatic treatment or liver transplantation in advanced stages^[9]. Therefore, the discovery of novel approaches targeting early-stage oxidative stress, particularly those derived from functional foods with potent antioxidant properties, holds great promise for preventing or ameliorating alcohol-induced liver injury^[10-11].

Garlic (*Allium sativum* L.) is one of the oldest known medicinal and culinary plants, and has been extensively documented to possess anti-inflammatory, antibacterial, immunomodulatory, and cardioprotective properties^[12-14]. Owing to its detoxifying and antioxidant activities, garlic has also been widely used to protect against liver injury, including ALD, metabolic-associated fatty liver disease, and drug-induced liver injury^[15-16]. Garlic contains a variety of bioactive compounds, among which organosulfur compounds are considered the principal contributors to its health benefits. Allicin, enzymatically formed from alliin by alliinase when garlic is chopped or crushed^[17], is the most representative organosulfur compound and is commonly incorporated into natural health products as a key functional ingredient^[18]. However, several limitations hinder its broader application, including its pungent odor, poor water solubility, low bioavailability, and instability under physiological conditions^[19-20]. Moreover, allicin has been associated with adverse effects, such as gastrointestinal irritation, raising concerns about its potential toxicity^[21-22].

Therefore, we developed a method to inactivate alliinase, thereby preventing the enzymatic conversion of alliin to allicin and enabling the production of an allicin-free garlic extract (AFGE). This study aimed to evaluate the protective effects of AFGE against alcohol-induced hepatotoxicity and to elucidate the underlying mechanisms. Our findings demonstrate that AFGE exerts antioxidant effects by targeting and activating PPAR γ , thereby mitigating alcohol-induced hepatic injury.

2. Materials and methods

2.1. Regents and materials

AFGE was manufactured and provided by Oululian Biotechnology Co., Ltd. (Guangdong, China). The reference standards alliin and allicin ($\geq 98\%$ purity) were purchased from Sichuan Weikeyi Biological Technology Co., Ltd. (WKQ-0000544; WKQ-0000155, Sichuan, China). Silymarin (Legalon® forte) was obtained from Madaus GmbH (Cologne, Germany). Methanol (HPLC-grade) was purchased from Thermo Fisher Scientific (A4524, MA, USA). Sodium carboxymethyl cellulose (CMC-Na, analytical grade) was obtained from Xilong Chemical Co., Ltd. (Guangdong, China). Absolute ethanol (analytical grade) was obtained from Beijing Chemical Industry Group Co., Ltd. (Beijing, China). Dimethyl sulfoxide (DMSO), phosphate-buffered saline (PBS), and trypsin-EDTA solution (0.25%, phenol red-free; cell culture grade) were all acquired from Beijing Solarbio Science & Technology Co., Ltd. (Beijing, China).

2.2 Preparation of AFGE

Unpeeled garlic bulbs (500 g) were steamed over boiling water for 10 minutes. After cooling to room temperature, the garlic was combined with 2.5 L of distilled water and homogenized using a blender in four 15-second intervals. The resulting mixture was then press-filtered through filtration fabric to obtain the garlic homogenate. The homogenate was subjected to ultrasonic extraction under 32 °C and 67 W for 75 minutes, followed by centrifugation at $13,800 \times g$ for 15 minutes. The supernatant was collected and concentrated using a rotary evaporator at 50 °C. The resulting concentrate was then freeze-dried to obtain a white powder, with an extraction yield of approximately 15%, referred to as AFGE.

2.3 HPLC assay

Twenty micrograms of AFGE were dissolved in 2 mL of distilled water and vortexed for 15 seconds. Following centrifugation at $9,600 \times g$ for 5 minutes, the sample solution was transferred into a glass vial, and 10 μ L of the sample was injected for HPLC analysis. The assay was conducted using a Waters e2695 HPLC system equipped with an ultra-violet detector (Waters W2489, Waters Technologies). Chromatographic separation was performed on a Purospher® STAR RP-18 column (250 mm $\times$ 4.6 mm, 5 μ mol/L, Merck, Germany) using a mobile phase consisting of water (A) and methanol (B), at a flow rate of 0.5 mL/min. For the quantification of alliin, a gradient elution was applied as follows: 0–10 minutes, 2% B; 10–15 minutes, 2%–30% B; 15–20 minutes, 30% B; 20–25 minutes, 30%–2% B; and 25–30 minutes, 2% B. For allicin detection, an isocratic elution with 75% B was used. To evaluate the reproducibility of the extraction procedure, three independently prepared batches of AFGE were analyzed under identical chromatographic conditions. Chromatogram data were acquired at a detection wavelength of 214 nm.

2.4 Animal experiments

To establish a model of acute alcohol-induced liver injury, mice were randomly divided into six groups ($n = 15/\text{group}$): vehicle control, alcohol, positive control (silymarin), and AFGE at doses of 0.5, 1, and 2 g/kg. The selected doses, particularly the highest dose (2.0 g/kg), were chosen based on prior rodent studies demonstrating that this dose was well tolerated^[23–24]. The vehicle control and alcohol groups received water

via intragastric administration, while the treatment groups were administered AFGE or silymarin (100 mg/kg) according to their weight. All administrations were conducted at a volume of 0.1 mL/10 g of body weight. After 29 days of treatment, the mice were fasted overnight with free access to water. One hour after the final gavage, a single dose of 50% alcohol (14 mL/kg) was administered to the alcohol, silymarin, and AFGE-treated groups to simulate human binge drinking^[25-26]. Sixteen hours after alcohol administration, mice were anesthetized with pentobarbital sodium (70 mg/kg, i.p.), and blood was collected via the abdominal aorta. The liver was then excised, and the mice were subsequently euthanized.

For the aged mouse model, forty-eight male ICR mice (eight months old) were housed individually, as aged mice are more aggressive than younger ones and prone to fighting. The mice were randomly assigned to four groups (n = 12/group) based on body weight: a vehicle control group and three experimental groups receiving AFGE at doses of 0.5, 1, and 2 g/kg. All treatments were administered by intragastric gavage daily for 30 days. At the end of the experiment, blood and liver tissues were collected following the procedures described above.

All animals were obtained from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China) and housed under controlled conditions: temperature at 23 ± 2 °C, relative humidity at $55\% \pm 10\%$, and a 12-h light/12-h dark cycle. All animal experiments were conducted in accordance with institutional guidelines and approved by the ethics committee. The study protocols complied with the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines.

2.5 Determination of biochemical indices in serum and liver

Serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and total triglyceride (TG) were measured using commercially available assay kits (100020013; 100020000; and 100020090, Biosino Bio, Beijing, China) according to the manufacturer's instructions, with an automated biochemical analyzer (Beckman Coulter AU480, Brea, CA, USA). Contents of malondialdehyde (MDA), protein carbonyl, glutathione (GSH), and catalase (CAT) in the serum and liver were also measured following the respective kit protocols. For the determination of total cholesterol (TC) and TG in liver tissue, samples were homogenized with 9-fold cold ethanol and centrifuged. The supernatants were collected for subsequent analysis. Hepatic TC and TG contents were normalized to the weight of liver tissue.

2.6 Histological analyses

For histology, liver tissues from seven randomly selected mice per group were processed for staining and subsequent analysis. Following dissection, a portion of the liver tissue was fixed in 4% paraformaldehyde overnight at room temperature, then dehydrated and embedded in paraffin. The tissue blocks were sectioned into 5 μ m-thick slices and stained with hematoxylin and eosin (H&E) to assess liver injury. For visualization of lipid accumulation, frozen liver sections were stained with Oil Red O. Histological features were examined under a light microscope and analyzed using SlideViewer software (3DHISTECH Ltd.,

Budapest, Hungary). The hepatic histopathological score was calculated as the sum of scores for steatosis (0–3), hepatocellular injury (0–4), and sinusoidal dilatation (0–3).

2.7 RNA-seq and data processing

Total RNA from livers was extracted and purified using TRIzol reagent (15596018CN, Thermo Scientific, MA, USA). RNA concentration and purity were assessed using a Bioanalyzer 2100 and an RNA 6000 Nano LabChip Kit (5067-1511, Agilent, CA, USA) to ensure suitability for downstream applications. After two rounds of purification, total RNA was fragmented and reverse-transcribed, and sequencing libraries were constructed according to the manufacturer's instructions. Paired-end sequencing (2 bp× 150 bp, PE150) was then performed on a NovaSeq 6000 platform (Illumina, San Diego, CA, USA) following the recommended protocol. Cleaned reads were aligned to the *Mus musculus* reference genome (GRCm39) using HISAT2. Transcript assembly and quantification of mRNA expression levels were performed using StringTie, and expression was normalized as fragments per kilobase of transcript per million mapped reads (FPKM). Differential gene expression between groups was analyzed using DESeq2. Genes with a false discovery rate (FDR) < 0.05 and an absolute fold change (FC) > 2 were considered differentially expressed and selected for further analysis. All RNA-seq samples met standard quality control checks. Detailed metrics are provided in Supplementary Table S1.

2.8 Enrichment analysis

Gene ontology (GO) enrichment analysis was performed to identify significantly overrepresented GO terms among the differentially expressed genes (DEGs), based on the Gene Ontology database (<https://www.geneontology.org/>). Pathway enrichment analysis was conducted using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database to explore the major biological functions and pathways associated with the DEGs. Gene set enrichment analysis (GSEA) was performed using GSEA software (v4.1.0) from the Molecular Signatures Database (MSigDB) to determine whether predefined gene sets showed significant differences in specific GO terms or KEGG pathways between the two groups. The criteria for significance were: $|\log_2FC| > 2$, $|NES| > 1$, nominal *P*-value < 0.05, and FDR *Q*-value < 0.25. All bioinformatic analyses were conducted using the OmicStudio platform (OmicShare, Guangzhou, China; <https://www.omicstudio.cn/tool>).

2.9 Quantitative Real-Time PCR

Total RNA was extracted from liver tissues using TRIzol reagent (CW0580S, CWbiotech, Beijing, China) according to the manufacturer's instructions. First-strand cDNA was synthesized from 2.5 µg of total RNA using a reverse transcription kit (AT341, TransGene, Beijing, China). Quantitative real-time PCR (qRT-PCR) was performed in a 20 µL reaction mixture containing 3 µL cDNA, 0.4 µL each of forward and reverse primers (10 µmol/L), 10 µL Hieff SYBR Green Master Mix (11201ES, Yeasen, Shanghai, China), and DEPC-treated water. Reactions were performed in 96-well plates using a CFX96 Real-Time PCR System (Bio-Rad,

CA, USA). Relative mRNA expression levels were calculated using the comparative Ct ($2^{-\Delta\Delta C_t}$) method, with *Actb* serving as the internal control. Gene-specific primers were designed by Sangon Biotech (Shanghai, China) and are listed in Table 1.

Table 1. Primers used for real time qPCR

Gene Primers	Sequences (5'-3')
<i>Gpx1</i> -Forward	TCTCTGAGGCACCACGATCCG
<i>Gpx1</i> -Reverse	ACCGAGCACCACCAGTCCAC
<i>Gpx3</i> -Forward	GGTCTGCTTGCCCTCCTAAATGCTG
<i>Gpx3</i> -Reverse	GTTCTCGCCTGGCTCCTGTTTG
<i>Gpx8</i> -Forward	TGGAGCCTTTCGCTGCCTACC
<i>Gpx8</i> -Reverse	GCTGTTGGTTCTCGGCTTCAGG
<i>Gsto2</i> -Forward	ACTACCTTCTTCGGCGGAGAC
<i>Gsto2</i> -Reverse	GATTCACGCAGTCAGCCAGTC
<i>Gsta1</i> -Forward	GGCTGACCAGGGTGGACATC
<i>Gsta1</i> -Reverse	GCTGCTGATTCTGCTCTTGAAGG
<i>Actb</i> -Forward	GTGCTATGTTGCTCTAGACTTCG
<i>Actb</i> -Reverse	ATGCCACAGGATTCCATACC

2.10 Drug target prediction

To explore potential pathways involved in the antioxidant effect of AFGE, candidate targets were predicted using the web-based tool omics and text-based target enrichment and ranking (OTTER) (<http://otter-simm.com/otter.html>)^[27]. The analysis was performed on the complete list of DEGs identified from our RNA-seq analysis using the keyword “oxidative stress” as a query keyword. OTTER calculates two scores for each gene: a Text Score based on the frequency of the keyword in related PubMed abstracts, and a PPI Score based on its connectivity in a protein-protein interaction network from STRING. These scores are integrated to generate a composite ranking. The analysis was run using all default parameters. Based on the integrated scores, OTTER generates a prioritized gene list. A PPI network for the top-ranked candidates is then created, which can be visualized and exported as an SDF file for their further validation.

2.11 Western blot analysis

Liver tissues and cultured cells were lysed using RIPA buffer (R0010, Solarbio, Beijing, China). Protein concentrations were quantified with a BCA Protein Assay Kit (CW0014S, CWbiotech, Beijing, China) and normalized to equal concentrations. Protein samples (30 µg) were denatured, separated on 10% SDS-polyacrylamide gels, and transferred onto 0.2 µmol/L nitrocellulose membranes (66485, Pall, NY, USA). Membranes were blocked with 5% non-fat milk and incubated overnight at 4 °C with a primary antibody against PPAR γ (1:1000; 60127-1-Ig, Proteintech, Wuhan, China). After washing, membranes were incubated with a horseradish peroxidase (HRP)-conjugated secondary antibody (1:5000; CWbiotech, Beijing, China) for 1 hour at room temperature. Protein bands were visualized using an eECL Western Blot Kit (CW0049S, CWbiotech, Beijing, China) and detected with an e-Blot Touch ImagerTM (e-Blot Life Science Co., Ltd., Shanghai, China). β -Actin (1:2000; AC038, ABclonal, Wuhan, China) was used as the internal loading control.

2.12 Cell culture and treatment

Human hepatocellular carcinoma HepG2 cells were obtained from Wuhan Pricella Biotechnology Co., Ltd. and authenticated by short tandem repeat (STR) profiling. Although HepG2 cells have a limited capacity for alcohol metabolism, they remain a well-established *in vitro* model for investigating downstream alcohol-induced oxidative stress and cell death^[28]. Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM; C11965500BT, Gibco, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS; 10099141C, Gibco, Grand Island, NY, USA) and 1% penicillin-streptomycin (P1400, Solarbio, Beijing, China), at 37 °C in a humidified incubator with 5% CO₂. HepG2 cells were pretreated with AFGE (150 and 300 µg/mL), alliin (5 and 10 µg/mL), or allicin (5 and 10 µg/mL) for 2 hours. Alcohol was then added to achieve a final concentration of 400 mmol/L and the cells were incubated for 24 hours.

To determine the PPAR γ dependence of AFGE's effects, HepG2 cells were preincubated with the selective PPAR γ antagonist T0070907 (HY-13202, MCE). The concentration of T0070907 (20 µmol/L) and the pre-incubation time (1 hour) were selected based on preliminary dose-response experiments, which identified 20 µmol/L as the optimal inhibitive concentration that robustly suppressed PPAR γ without cytotoxicity (Fig. S3A and C). HepG2 cells were pre-treated with T0070907 and subsequently co-incubated with AFGE (300 µg/mL) and alcohol (400 mmol/L) for 24 hours. Cell viability was assessed using the CCK-8 assay, and PPAR γ protein abundance was analyzed by Western blotting.

2.13 CCK-8 assay

After 24 hours of alcohol exposure, CCK-8 solution (1:10 dilution; BS350A, Biosharp, Hefei, China) was added to each well, and cells were incubated at 37 °C for 45 minutes. The absorbance was then measured at 450 nm using a microplate reader. Cell viability was calculated as a percentage relative to the control group. All experiments were independently repeated three times.

2.14 TUNEL staining

Experimental cells were fixed and permeabilized with 4% formaldehyde. TUNEL staining was then performed using a One-Step TUNEL Apoptosis Assay Kit (C1086, Beyotime, Jiangsu, China) according to the manufacturer's instructions. Cell nuclei were counterstained with Hoechst 33258 (1 µL of 1 mg/mL solution; C0021, Solarbio, Beijing, China). Apoptotic cells induced by alcohol exposure were identified by the co-localization of TUNEL and Hoechst 33258 signals. All slides were visualized using a fluorescence microscope.

2.15 Detection of ROS generation

To assess ROS production, HepG2 cells were trypsinized, washed with PBS, and incubated with 10 µmol/L DCFH-DA (S0033S, Beyotime, Jiangsu, China) in PBS at 37 °C for 30 minutes. Following incubation, cells were centrifuged at 1500 rpm (corresponding to 312 × g) for 5 minutes, washed three times with PBS,

and the fluorescence intensity was measured using a microplate reader at an excitation wavelength of 488 nm and an emission wavelength of 525 nm. Experiments were performed in triplicate.

2.16 Molecular docking

Molecular docking was conducted using CDOCKER within Discovery Studio 2016 (BIOVIA, San Diego, CA, USA) to evaluate ligand binding to PPAR γ . The 3D structure of human PPAR γ (PDB ID: 6T1S; <https://doi.org/10.2210/pdb6T1S/pdb>) was obtained from the Protein Data Bank and used as the receptor. The chemical structures of alliin and allicin were retrieved from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>) and used as ligands. Docking simulations identified the pose with the lowest -CDOCKER energy score as the optimal binding conformation. The ligand–receptor interactions were visualized in 2D and 3D using Discovery Studio and PyMOL (version 2.5, Schrödinger, LLC, New York, USA), respectively.

2.17 Statistical analysis

All data are shown as mean $\pm$ SEM. Data normality was assessed by the Shapiro–Wilk test, and homogeneity of variance was evaluated using the Brown–Forsythe test (GraphPad Prism v8.0.2). For multi-group comparisons meeting normality and homoscedasticity, one-way ANOVA with Dunnett’s post hoc test was used; otherwise, the Kruskal–Wallis test with Dunn’s post hoc test was applied. In all cases, P -values < 0.05 were considered statistically significant. The statistical tests applied to each experiment were indicated in the corresponding figure legends.

3. Results and discussion

3.1 Characterization of the composition of AFGE

The instant powder of AFGE was provided by Oululian Co., Ltd. To determine the contents of alliin and allicin in AFGE, an HPLC method was developed for the simultaneous quantification of both compounds. Fig. 1 presents the HPLC chromatograms of AFGE (Fig. 1A and B) and authentic standards (Fig. 1C and D), acquired using two different mobile phase systems: water and methanol. Under optimized chromatographic conditions, alliin and allicin were well separated from other components. The content of alliin in AFGE was quantitatively determined to be 3.53%, based on a standard curve constructed from peak area versus concentration of the alliin standard. In contrast, allicin was not detected in AFGE, indicating that alliin was not converted to allicin during the preparation process. These results are consistent with the information provided by the manufacturer. Analysis of three independent AFGE batches demonstrated high reproducibility, exhibiting consistent chromatograms (Fig. S1) with minimal variation in both chromatographic retention time (RSD = 0.21%) and alliin content (inter-batch CV = 3.30%), as detailed in Table 2.

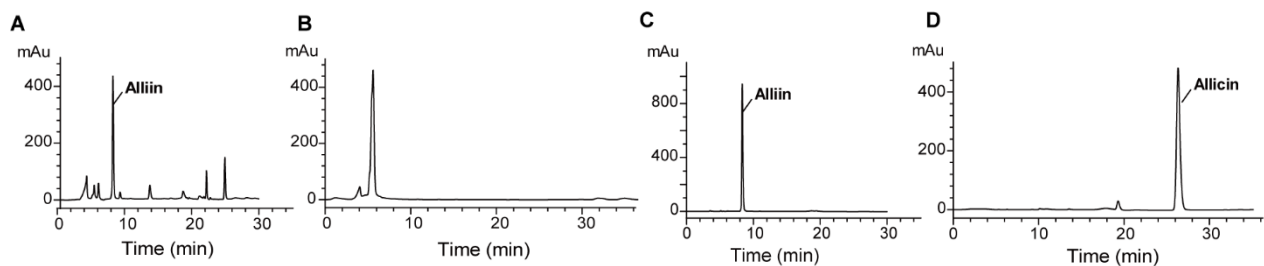


Fig. 1. HPLC-UV chromatograms of AFGE (top) and authentic standards (bottom) at 214 nm. (A, C) Gradient elution with 2%–30% methanol in water. (B, D) Isocratic elution with 75% methanol in water.

Table 2. Retention-time stability and peak-area precision for three HPLC peaks across three batches of AFGE.

	Peak 1 (Alliin)	Peak 2	Peak 3
Retention time (min)			
Batch 1	8.291	22.199	24.937
Batch 2	8.309	22.192	24.915
Batch 3	8.326	22.182	24.907
Mean	8.309	22.191	24.920
SD	0.0176	0.0086	0.0153
RSD (%)	0.21	0.04	0.06
Peak-area			
Batch 1	82.378	13.488	27.190
Batch 2	85.289	16.011	28.374
Batch 3	88.001	17.129	30.276
Mean	85.223	15.543	28.613
SD	2.8118	1.865	1.557
RSD (%)	3.30	12.00	5.44

3.2 AFGE attenuates alcohol-induced liver injury in mice

To evaluate the hepatoprotective effects of AFGE, mice were administered varying doses of AFGE or the positive control drug silymarin for 30 days, followed by induction of acute liver injury with 50% alcohol (Fig. 2A). Throughout the experimental period, no significant differences in body weight were observed among all groups, nor in liver weight after the final alcohol administration. These findings indicate that AFGE is well tolerated at these doses (Fig. 2B, C). Following a single dose of alcohol, the liver injury model was successfully established, as evidenced by markedly elevated serum levels of ALT, AST, and TG compared with the control group (Fig. 2D–F). Consistently, histopathological analysis revealed that alcohol exposure induced microvesicular steatosis predominantly around the centrilobular vein, mild hepatocyte ballooning, sporadic acidophilic bodies, and moderate sinusoidal dilatation, in contrast to the normal liver morphology observed in the control group (Fig. 2G). AFGE, although not as effective as silymarin, significantly reduced serum ALT, AST, and TG levels without a clear dose-response relationship, while it markedly alleviated alcohol-induced histopathological changes in a dose-dependent manner (Fig. 2D–G). In addition, AFGE dose-dependently reduced hepatic lipid accumulation following alcohol exposure, as demonstrated by decreased hepatic TC and TG contents (Fig. 2H, I) and lower Oil Red O staining scores compared with the alcohol group (Fig. 2L). Notably, the mitigative effect of AFGE at 2 g/kg on alcohol-induced hepatic injury and steatosis was comparable to that of the positive control, silymarin.

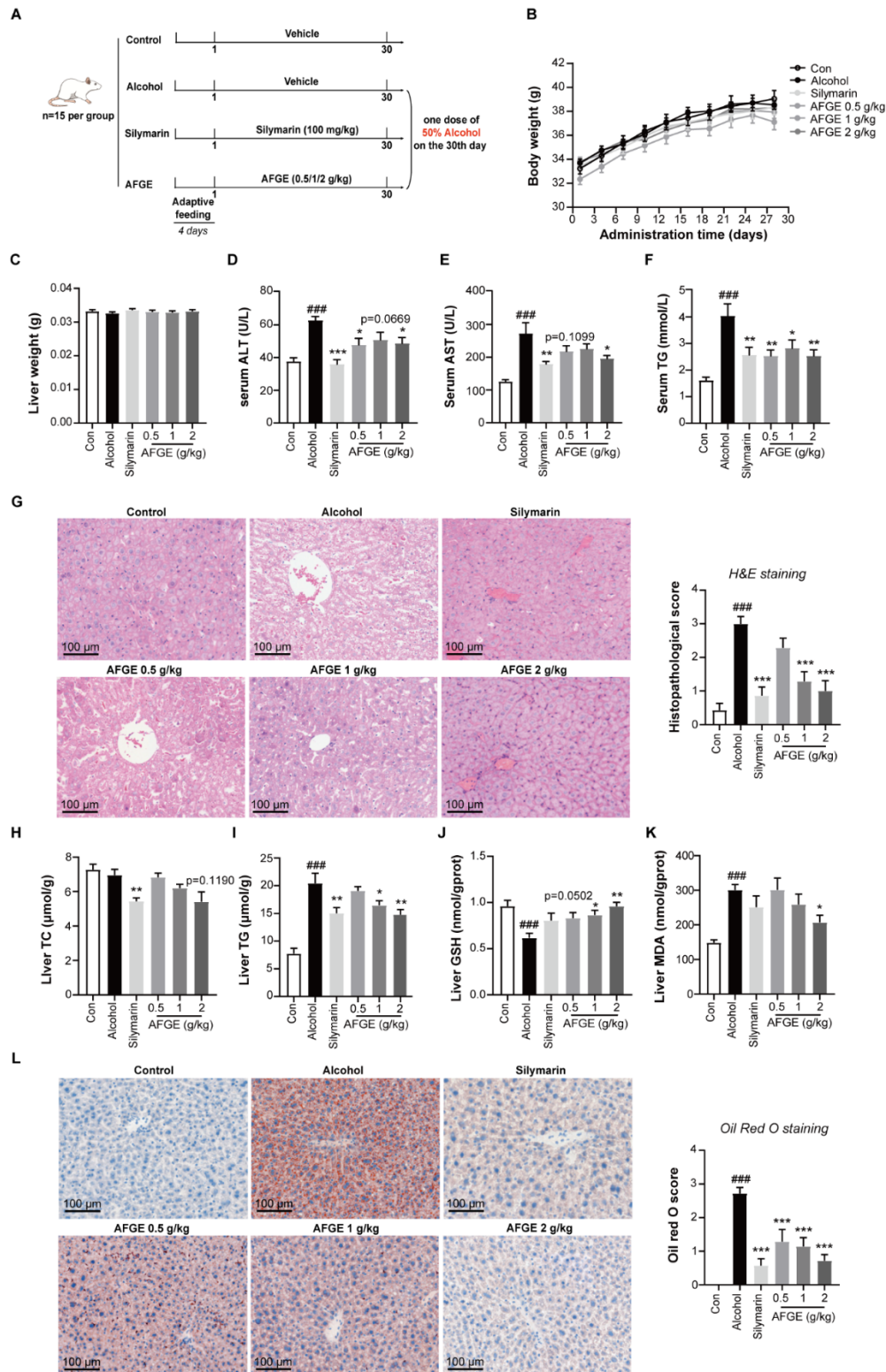


Fig. 2 AFGE attenuates alcohol-induced acute liver injury in mice. (A) Schematic diagram of the experimental design. ICR mice were administered vehicle, silymarin (100 mg/kg) or AFGE (0.5, 1, or 2 g/kg), by gavage for 30 days, followed by an intragastric dose of 50% alcohol (14 mL/kg) to induce acute liver injury (n = 15). (B) Body weight changes during the treatment period. (C–F) Liver weight (C), and serum levels of alanine aminotransferase (ALT, D), aspartate aminotransferase (AST, E), and triglyceride (TG, F) following alcohol administration. (G) Representative hematoxylin and eosin (H&E) staining of liver sections (left), with quantification of histopathological changes (right, n = 7). Scale bar = 100 μ m. (H–K) Hepatic contents of total cholesterol (TC, H), TG (I), glutathione (GSH, J), and malondialdehyde (MDA, K). (L) Representative Oil Red O-stained liver sections (left) and quantification of hepatic lipid accumulation (right, n = 7). Scale bar = 100 μ m. Data are expressed as mean $\pm$ SEM. Statistical significance was determined by one-way ANOVA with Dunnett's post hoc test (C, D, F, I, K) or Kruskal–Wallis test for nonparametric data (E, G, H, J, L). ### $P < 0.001$ vs. control group; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. alcohol group.

To further explore the potential mechanisms that drive the hepatoprotective effects of AFGE, biochemical markers of oxidative stress were assessed, given the pivotal role of oxidative damage in alcohol-induced liver injury^[29]. AFGE greatly increased hepatic GSH contents (Fig. 2J), which was even higher than that of the silymarin group and reduced MDA contents (Fig. 2K), indicating a dose-dependent attenuation of alcohol-induced oxidative stress. Collectively, these findings suggest that AFGE alleviates acute alcohol-induced liver injury, at least in part, through its antioxidant activity *in vivo*. Notably, the 2 g/kg dose of AFGE demonstrated the most pronounced protective effects against both hepatocellular damage and hepatic lipid accumulation.

3.3 AFGE exhibits antioxidant activity in aged mice

Age-related increases in oxidative stress are well-documented and are considered to result from the accumulation of free radicals and a decline in endogenous antioxidant capacity^[30-31]. To further validate the antioxidant potential of AFGE, an aged mouse model (8 months old) was employed. During the 30-day continuous drug administration period, no significant differences in body weight (Fig. 3A) or liver weight (Fig. 3B) were observed among all groups, indicating that AFGE was well tolerated in aged mice at the tested doses. The biomarkers of oxidative stress in the serum and liver were detected to verify our hypothesis. AFGE treatment reduced serum MDA contents by 26.04%, 0.82%, and 31.36%, respectively, suggesting a decrease in lipid peroxidation (Fig. 3C). Compared with the control group, serum contents of GSH and CAT were moderately elevated following AFGE treatment, indicating its potential to enhance antioxidant enzyme activity in aged mice (Fig. 3C). Similarly, AFGE dose-dependently enhanced hepatic antioxidant defenses, as evidenced by decreased levels of MDA and protein carbonyls, along with increased hepatic GSH and CAT contents (Fig. 3D). Collectively, these data suggest that AFGE exerts antioxidant effects by increasing the levels of antioxidant enzymes and strengthening the endogenous antioxidant system, thereby protecting the liver from oxidative stress.

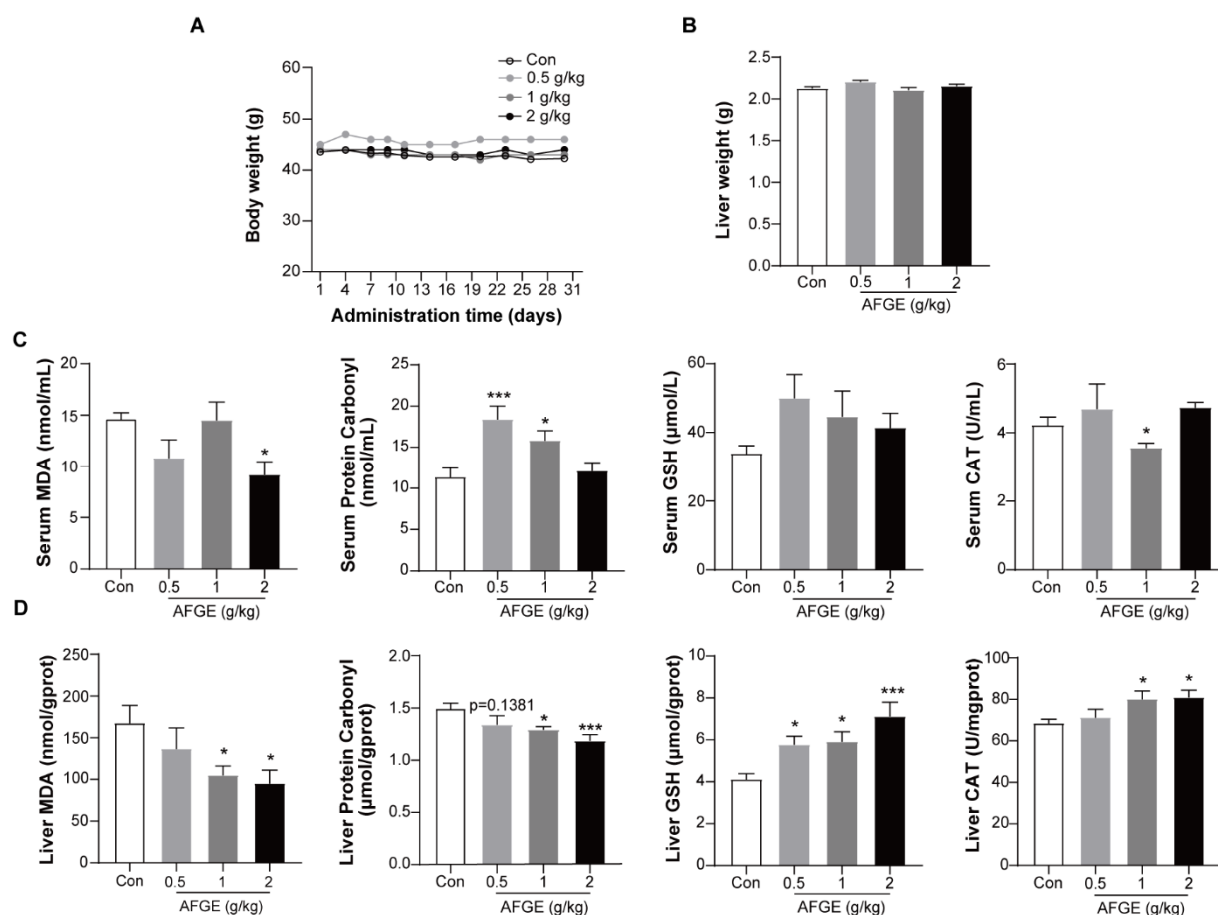


Fig. 3 AFGE reduces oxidative stress in aged mice. Eight-month-old ICR mice were administered AFGE (0.5, 1, or 2 g/kg) by gavage for 30 days ($n = 12$). (A) Body weight changes during the treatment period. (B) Liver weight of the mice in the indicated groups. (C, D) Serum (C) and hepatic (D) contents of malondialdehyde (MDA), protein carbonyl, glutathione (GSH), and catalase (CAT). Data are presented as mean $\pm$ SEM. Statistical significance was determined by one-way ANOVA with Dunnett's post hoc test (A-D). * $P < 0.05$, *** $P < 0.001$ vs. control group.

3.4 Transcriptome analysis of livers from mice treated with or without AFGE

To elucidate the molecular mechanisms underlying the antioxidant effects of AFGE in alcohol-induced hepatotoxicity, RNA sequencing was performed. The highest AFGE dose (2 g/kg), which exhibited the most potent antioxidant activity, was selected for comparison with the alcohol-treated group. Each group included three biological replicates. Principal component analysis (PCA) and inter-sample correlation analysis showed that the RNA-seq data were of high quality and revealed distinct transcriptional profiles among the experimental groups (Fig. S4). Following data normalization, DEGs were identified based on a fold change > 2 and $P < 0.05$. Consistent with its beneficial effects in mice, AFGE downregulated genes associated with apoptosis and alcohol metabolism, while upregulating those involved in antioxidant responses and inhibition of apoptosis (Fig. 4A). DEGs were further functionally annotated and enriched using bioinformatics methods, including GO annotation, KEGG and GSEA^[32]. GO enrichment analysis of DEGs between the AFGE-treated and alcohol groups revealed significant enrichment of biological processes and pathways related to immune response, antioxidant system, lipid metabolism, detoxification and alcohol metabolism, as shown in Fig. 4B. Similarly, KEGG pathway enrichment analysis revealed significant enrichment of the NF- κ B signaling pathway, apoptosis, glutathione metabolism, and the PPAR signaling pathway, highlighting potential

pathways and molecular mechanisms underlying the effects of AFGE (Fig. 4C). GSEA revealed alterations in key pathogenic processes in ALD, including alcohol metabolism, oxidative stress, inflammation, and cell death regulation. AFGE treatment significantly upregulated gene sets related to both enzymatic activity and ROS metabolism process, while downregulating pathways linked to inflammatory response and programmed cell death (Fig. 4D). Given its enhanced antioxidant activity, further analysis focused on redox-related pathways, revealing that AFGE upregulated antioxidant enzyme activity and downregulated pathways associated with ROS generation, potentially accounting for its antioxidant effects observed in mice (Fig. 4E). Notably, the gene set related to glutathione peroxidase (GPX) activity ranked the highest, this set represents enzymes whose antioxidation effect relies on GSH to scavenge hydrogen peroxide (H_2O_2) produced within the cells. Moreover, the substrate of GPX, GSH, increased in mice livers following AFGE treatment (Fig. 2J and 3D), suggesting the involvement of the GSH–GPX axis in the antioxidant effects of AFGE. Collectively, transcriptomic analysis revealed that AFGE modulates alcohol metabolism and enhances endogenous antioxidant defense system, particularly through GSH-mediated processes.

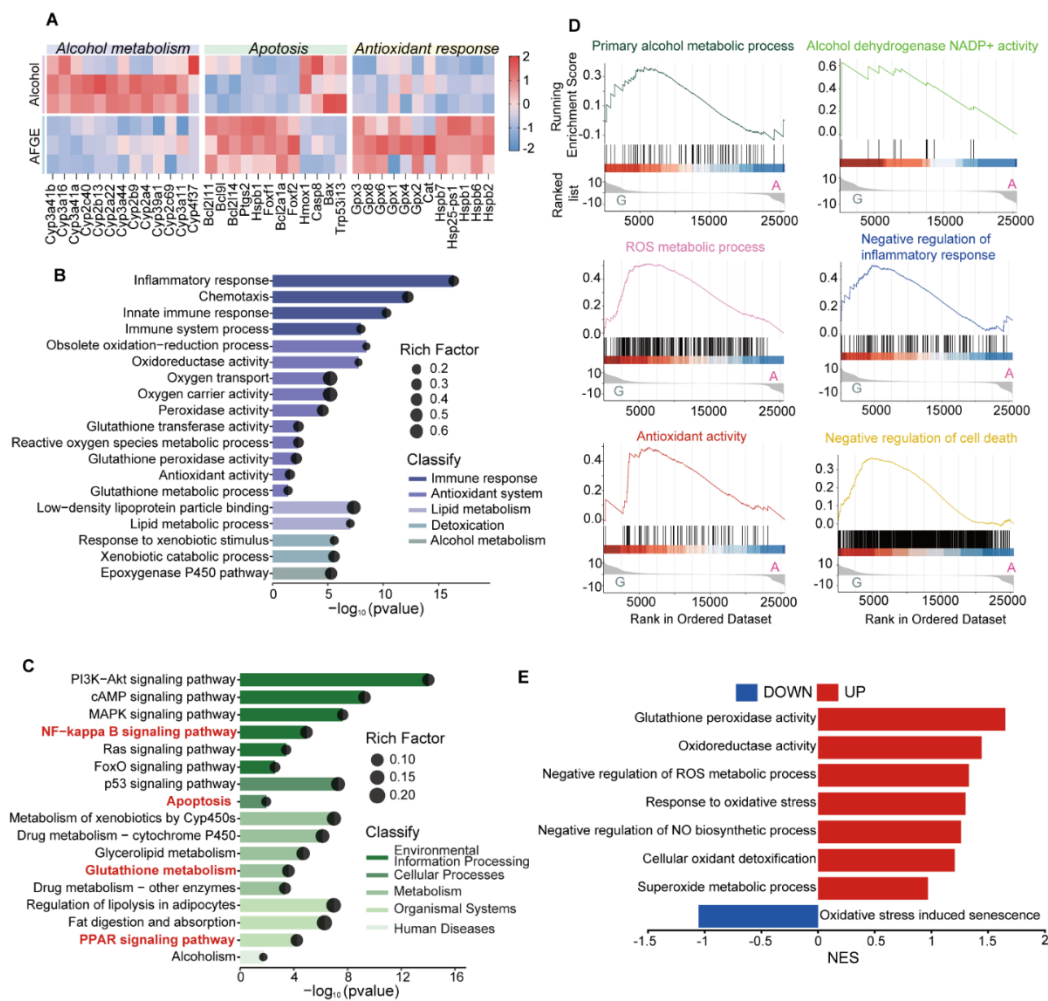


Fig. 4 In-depth transcriptomic analysis of liver tissues from the alcohol and AFGE (2 g/kg) groups. (A) RNA-seq heatmap showing differentially expressed genes (DEGs) between the alcohol group and the AFGE-treated group ($n = 3$). Genes involved in alcohol metabolism, apoptosis, and antioxidant response are highlighted. (B, C) Gene Ontology (GO) functional enrichment analysis (B) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis (C) of DEGs. (D, E) Gene set enrichment analysis (GSEA) based on GO terms: (D) pathways associated with the pathogenesis of alcohol-induced fatty liver disease; (E) pathways related to oxidative stress. NES, Normalized Enrichment Score. “A” indicates the alcohol group; “G” indicates the AFGE treatment group.

3.5 Identification of hub genes associated with antioxidant activity of AFGE

Given that endogenous antioxidant system comprises both enzymatic and non-enzymatic components, a bioinformatic tool named omics and text based target enrichment and ranking (OTTER) was employed to identify the hub genes regulated by AFGE to exert its antioxidant function^[27]. The term “oxidative stress” was defined as the keyword to comprehensively retrieve all the available PubMed abstracts associated with DEGs. OTTER then prioritized the most relevant genes by integrating the text-mining and protein-protein interaction (PPI) network scores and visualized their interactions in a PPI network (Fig. 5A). *Hmox1*, *Gpx3* and *Gclc*—genes closely involved in the GSH metabolism process—ranked among the top three candidates (Fig. 5B). *Hmox1* and *Gclc* are established downstream targets of the Nrf2 transcription factor and play important roles in mitigating oxidative stress induced by exogenous stimuli^[33]. Although AFGE treatment led to increased *Hmox1* expression, *Gclc* was significantly downregulated according to RNA-seq data, suggesting that AFGE may not exert its antioxidant effects through activation of the Nrf2 pathway and its downstream detoxification targets. *Gpx3* encodes glutathione peroxidase 3 (GPX3), a highly efficient antioxidant enzyme that participates in GSH-mediated redox reactions and catalyzes the reduction of organic hydroperoxides and H₂O₂ using GSH as a substrate^[34]. Furthermore, in the PPI network (Fig. 5C), GPX3 was functionally associated with *Gsto2* and *Gsta1*, two members of the glutathione S-transferase (GST) family. These enzymes catalyze the conjugation of GSH to electrophilic compounds and have been implicated in the pathogenesis of ALD^[6]. In addition to the OTTER analysis, GSEA revealed that the gene sets related to glutathione peroxidase activity and glutathione transferase activity were significantly enriched and upregulated following AFGE treatment (Fig. 5D). *In vivo*, eight phylogenetically related but structurally distinct GPX isoforms have been identified, all of which play critical roles in scavenging ROS^[35]. To corroborate the above results obtained from bioinformatics analysis, qRT-PCR analysis was conducted. The results showed that the mRNA expressions of *Gpx1*, *Gpx3*, and *Gpx8* were upregulated following AFGE treatment compared with the alcohol group (Fig. 5F), with *Gpx3* exhibiting the most pronounced increase. Similarly, the transcript levels of *Gsto2* and *Gsta1* were significantly elevated in response to the 2 g/kg AFGE intervention. These results suggest that AFGE may enhance the GSH detoxification pathway by upregulating *Gpx3*, *Gsto2*, and *Gsta1*, thereby mitigating alcohol-induced oxidative damage in the liver.

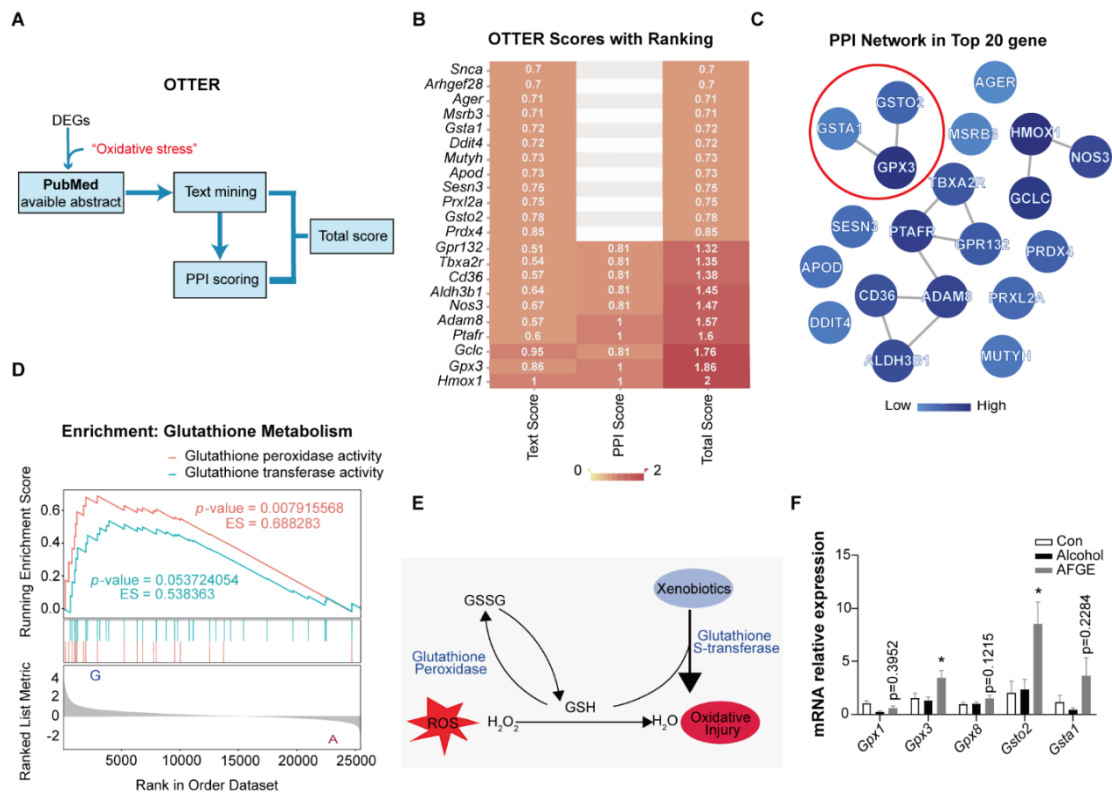


Fig. 5 Identification of key genes involved in the antioxidant effect of AFGE in alcohol-induced liver injury. (A) Schematic workflow of the omics and text-based target enrichment and ranking (OTTER) computational tool. (B) Ranking of differentially expressed genes (DEGs) based on total scores calculated by OTTER using RNA-seq data from Fig. 4, with “oxidative stress” as the keyword. (C) The protein–protein interaction (PPI) network among the top-ranked gene products was visualized using STRING, with protein names indicated in uppercase. (D) GSEA of glutathione metabolism-related pathways including the terms of “glutathione peroxidase activity” and “glutathione transferase activity”. ES, enrichment score; A, alcohol group; G, AFGE group. (E) Schematic overview of the physiological roles and metabolism of glutathione (GSH) in antioxidant defense. ROS, reactive oxygen species; GSSG, glutathione oxidized. (F) Quantitative real-time PCR (qRT-PCR) analysis of the indicated genes (n = 8). Data are presented as mean ± SEM. Statistical significance in (F) was determined by one-way ANOVA followed by Dunnett’s post hoc test. * $P < 0.05$ vs. alcohol group.

3.6 AFGE targets PPAR γ to exert antioxidant effects

Next, we explored the direct targets of AFGE that might account for the upregulation of *Gpx3*, *Gsto2*, and *Gsta1*. The most likely upstream signal for the transcriptional activation of these genes would be transcription factors (TFs), as they coordinate gene networks with similar functions and are known to be regulatable targets. In particular, nuclear receptors such as farnesoid X receptor (FXR), liver X receptor (LXR), and PPARs have been reported to regulate genes involved in antioxidant defense^[36–38]. A Venn diagram was constructed to identify overlapping TFs upstream of *Gpx3*, *Gsto2*, and *Gsta1* (Fig. 6A). A total of 424 TFs were screened using Cytoscape software, and the top candidates with the highest regulatory potential are presented in Fig. 6B. Among them, PPAR γ emerged as the most likely candidate, given its well-established role in cellular antioxidant defense, its known inactivation by alcohol exposure, and its involvement in various liver diseases^[39–41]. Having nominated PPAR γ as a putative target, we next investigated whether AFGE activates this pathway *in vivo*. GSEA confirmed a significant enrichment of the PPAR signaling pathway following AFGE treatment (Fig. 6C). Concordantly, hepatic PPAR γ protein expression increased (Fig. 6D), and its target genes, including *Cd36*, *Lpl*, *Slc2a4*, *Cidea*, *Fabp3*, *Apoa5*, and *Cpt1b*, were

upregulated (Fig. 6E). Taken together, all data show that AFGE engages hepatic PPAR γ signaling *in vivo*, supporting the involvement of PPAR γ in its hepatoprotective effects.

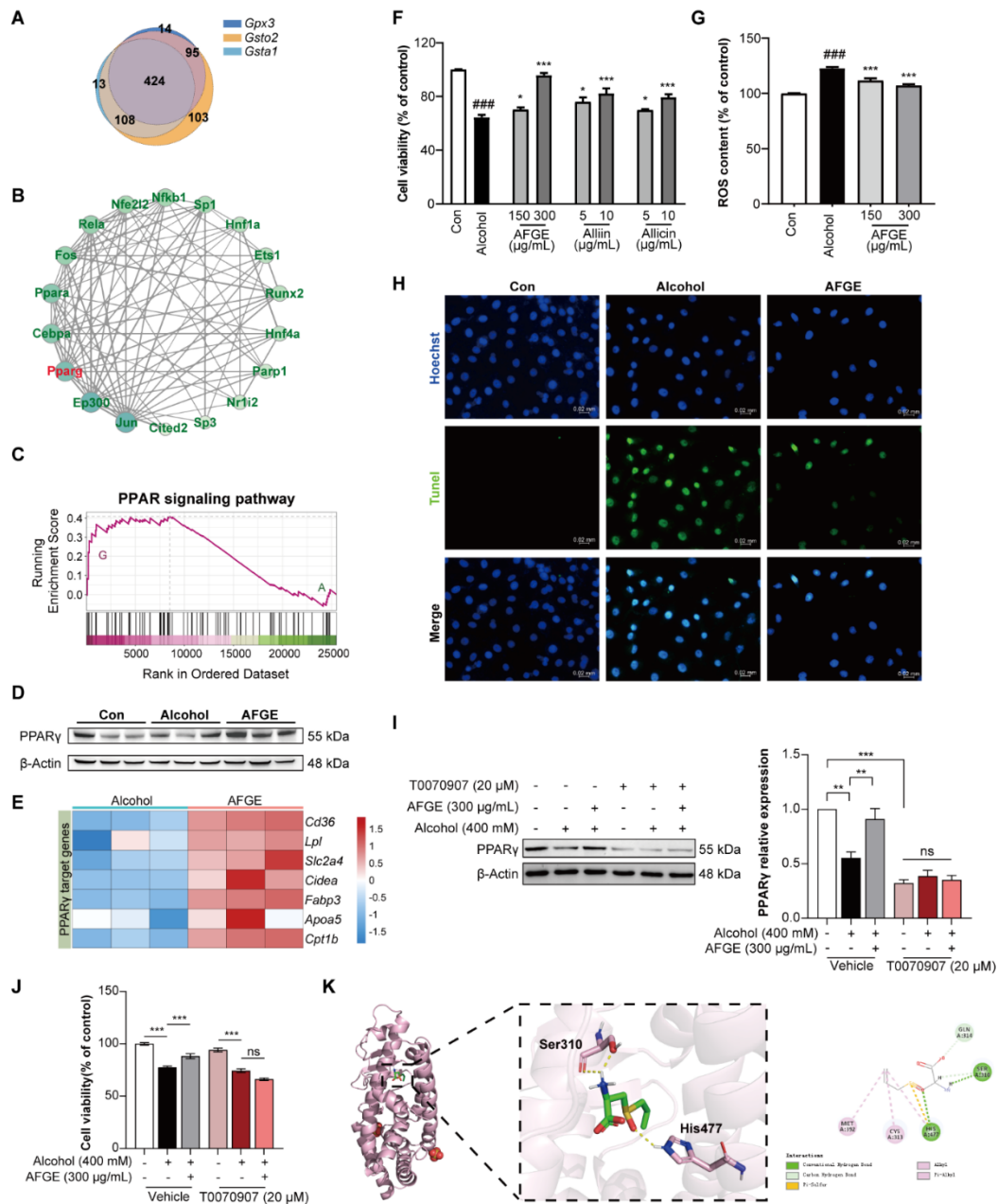


Fig. 6 PPAR γ is identified as a direct target of AFGE that transcriptionally activates antioxidant genes. **(A)** Venn diagram showing the overlap of predicted upstream transcription factors regulating *Gpx3*, *Gsto2* and *Gsta1*. **(B)** Cytoscape network visualization of candidate transcription factors identified in (A). **(C)** GSEA enrichment plot of the PPAR signaling pathway based on differentially expressed genes (DEGs). A, alcohol group; G, AFGE group. **(D)** Western blot analysis of PPAR γ in liver tissues from the indicated groups (n = 5). **(E)** the expression of PPAR γ target genes between the alcohol and the AFGE treatment groups (n = 3). **(F)** Cell viability of HepG2 cells treated with AFGE (150 or 300 μ g/mL), alliin (5 or 10 μ g/mL), or allicin (5 or 10 μ g/mL), followed by 400 mmol/L alcohol treatment for 24 hours. **(G)** Intracellular reactive oxygen species (ROS) levels in alcohol-treated HepG2 cells with or without AFGE (150 or 300 μ g/mL) for 24 hours. **(H)** TUNEL staining of HepG2 cells pretreated with AFGE (300 μ g/mL) followed by 400 mmol/L alcohol exposure for 24 hours. **(I)** Western blot analysis of PPAR γ in HepG2 cells from the indicated groups. **(J)** Cell viability of HepG2 cells with AFGE (300 μ g/mL) in the presence or absence of T0070907 (20 μ mol/L), followed by 400 mmol/L alcohol treatment for 24 hours. **(K)** Two-dimensional interaction map between PPAR γ and alliin, with interaction types are color-coded as indicated; and hydrogen bonds to Ser310 and His477 are indicated. Data are presented as mean $\pm$ SEM from three independent experiments. Statistical analysis in (F–J) was performed using one-way ANOVA followed by Dunnett's post hoc test. For (E and F), $^{###} P < 0.001$ vs. control group; $^{*} P < 0.05$, $^{***} P < 0.001$ vs. alcohol group. For (H and I), $^{*} P < 0.05$, $^{**} P < 0.01$, $^{***} P < 0.001$ for the indicated pairwise comparisons (as marked in the figure).

3.7 AFGE protects HepG2 cells against alcohol-induced injury in a PPAR γ -dependent manner

In addition to *in vivo* evaluation of hepatoprotective effects, the beneficial effect of AFGE was further validated using an *in vitro* alcohol-induced cellular injury model^[42]. On the basis of a pilot study involving escalating doses of alcohol (0, 50, 100, 200, 300, 400 mmol/L) and previous reports^[43], 400 mmol/L alcohol for 24 hours was selected to induce oxidative injury in HepG2 cells (Fig. S2A). Exposure to alcohol at a concentration of 400 mmol/L reduced cell viability (Fig. 6F), increased ROS production (Fig. 6G), and induced cell death (Fig. 6H). AFGE treatment (150 and 300 μ g/mL) effectively counteracted those deleterious effects by preventing alcohol-induced reduction in cell viability, suppressed ROS production and decreased cell death, as indicated by a lower number of TUNEL-positive cells (Fig. 6E–G). Furthermore, AFGE itself showed no cytotoxicity at these concentrations (Fig. S2B). Given that AFGE contains alliin but not allicin, we investigated whether alliin serves as the bioactive component. Indeed, alliin administered at equivalent doses also significantly restored cell viability. Moreover, the protective effect of AFGE on cell viability was superior to that of allicin alone (Fig. 6F).

To further assess whether the hepatoprotective effect of AFGE was PPAR γ -dependent, we next pretreated HepG2 cells with the selective PPAR γ antagonist T0070907 (20 μ mol/L) prior to AFGE administration^[44]. Suppression of PPAR γ markedly attenuated the AFGE-mediated improvement in cell viability (Fig. 6I and J), indicating that AFGE exerts its cytoprotective actions, at least in part, through activation of PPAR γ .

Molecular docking simulations were then performed using the CDOCKER algorithm to predict the interaction and explore the binding mode. Forty conformations of alliin bound to PPAR γ were generated, and the optimal pose was selected based on the lowest CDOCKER interaction energy. The analysis revealed that alliin formed direct hydrogen bonds with Ser310 and His477, hydrophobic interactions with Met392 and Cys313, and a π -sulfur interaction with His477 (Fig. 6K). It has been reported that alliin can be converted to allicin by alliinase^[45]. Although allicin exhibited protective effects against alcohol-induced cellular damage (Fig. 6F), molecular docking showed no binding affinity between allicin and PPAR γ . Consistently, only alliin, but not allicin, increased PPAR γ protein expression in alcohol-treated HepG2 cells (Fig. S3B and D).

Taken together, these findings indicate that alliin is the principal active component in AFGE responsible for binding to PPAR γ and promoting the transcriptional activation of downstream antioxidant genes and protecting liver against alcohol-induced injury.

4. Discussion

Liver, as the primary organ responsible for alcohol metabolism and detoxification, is particularly susceptible to alcohol-induced oxidative stress^[46–49]. It is well established that mitigating oxidative stress represents a promising strategy for the prevention and treatment of ALD^[50]. Although pharmacological agents such as silymarin are currently available for ALD management, concerns regarding their potential side effects have driven the search for safer alternatives. In this context, functional foods with potent antioxidant properties

and favorable safety profiles have emerged as attractive candidates for ALD intervention^[51-52]. Here, we successfully prepared a novel garlic extract (AFGE) devoid of allicin by inactivating alliinase and demonstrated that this extract effectively alleviates alcohol-induced liver injury and markedly reduces oxidative stress, primarily through activation of PPAR γ by its major active component, alliin.

Heating garlic at 100 °C for 10 minutes effectively inactivated alliinase, the enzyme responsible for converting alliin to allicin, while preserving alliin in the extract even after mechanical disruption^[53-55]. As expected, HPLC analysis confirmed that AFGE was free of allicin, and quantitative analysis revealed that the content of alliin was 3.53%. Notably, this represents a three-fold increase compared to dried garlic powder, which generally contains approximately 1% alliin^[56]. This substantial enrichment was achieved through our optimized extraction and concentration process targeting sulfur-containing compounds, and AFGE finally provides a substantially alliin-enriched, allicin-free preparation.

A single binge dose of alcohol administered via gavage is a well-established method for inducing acute alcoholic liver injury in mice^[26, 57]. Following exposure to a hepatotoxic dose of alcohol, characteristic histopathological changes including hepatocellular damage, steatosis, and sinusoidal dilatation are observed in liver tissue. Consequently, serum ALT and AST, biomarkers of hepatic injury^[3], were significantly increased. AFGE markedly ameliorated alcohol-induced liver injury and reduced hepatic lipid accumulation, as demonstrated by improved histological features (H&E staining), decreased serum ALT and AST levels, reduced lipid deposition (Oil Red O staining), and lower hepatic TG and TC levels. While hepatic histopathology improved in a dose-dependent manner after AFGE treatment, some serum markers (ALT, AST and TG) did not exhibit a strictly monotonic dose-response. This discrepancy may stem from fundamental differences between local tissue responses and circulating biomarkers. Histological assessment directly reflects the extent of liver injury and repair, which may correlate more closely with local drug exposure and effect. In contrast, serum marker levels are also influenced by other factors, such as their release and clearance kinetics, and can vary more between individual animals at a single endpoint. Furthermore, AST is not liver-specific^[58], and serum TG is subject to systemic metabolic regulation^[59], both of which may further obscure dose-dependent trends. Given the critical role of oxidative stress in the pathogenesis of ALD^[60-61], the hepatoprotective effects of AFGE are likely attributed, at least in part, to its capacity to enhance endogenous antioxidant defenses. Consistent evidence from the aged mouse model, the alcohol-induced HepG2 cell model, employed to investigate downstream injury pathways, and corroborating bioinformatic analyses indicates that AFGE exerts hepatoprotection primarily through direct antioxidant actions. Previous studies have demonstrated the protective effects of garlic oil against alcohol-induced liver injury^[62]. However, unlike AFGE used here, garlic oil primarily contains diallyl trisulfide and diallyl disulfide, degradation products of allicin, as its major bioactive components^[63], rather than alliin. Notably, the well-documented antioxidant properties of alliin likely underpin the observed antioxidant effects of AFGE^[64-65].

GSH, a non-enzymatic antioxidant, plays a pivotal role in the cellular antioxidant defense system by scavenging ROS and detoxifying xenobiotics^[66]. AFGE notably increased hepatic GSH content in both

experimental mice models, consistent with previous studies involving aqueous garlic extract^[13, 67], aged garlic extract^[68], and dietary garlic powder^[69]. GO enrichment and GSEA revealed significant enrichment of glutathione metabolic processes and antioxidant enzymes associated with glutathione function. Further investigation using bioinformatics tools identified *Gpx3*, *Gsto2*, and *Gsta1* as the hub genes mediating the antioxidant effects of AFGE. GPX3, a member of the glutathione peroxidase family, catalyzes the reduction of H₂O₂ and lipid peroxides by utilizing GSH as an electron donor^[70]. GSTs, as phase II detoxification enzymes, play a vital role in maintaining intracellular redox balance and protecting cells from oxidative damage^[71]. Previous studies have demonstrated that various forms of garlic products including allicin can enhance GPX activity^[12, 72-75], however, the underlying molecular mechanisms remain largely unclear. Moreover, *Gpx3* has been reported to exert antioxidant effects downstream of PPAR γ activation, suggesting it functions as a PPAR γ -responsive gene^[76]. However, its specific role in protecting against alcohol-induced oxidative stress has not been previously documented. In the present study, transcriptomic analysis in combination with molecular docking yielded findings consistent with PPAR γ acting as a potential upstream regulator of *Gpx3*, *Gsto2*, and *Gsta1*, and as a direct target of alliin, the major active compound in AFGE. Docking results revealed that alliin forms stable hydrogen bonds with PPAR γ at residues Ser310 and His477, and treatment with AFGE or alliin markedly increased PPAR γ protein expression, supporting that alliin may function as a PPAR γ agonist. Beyond regulating antioxidant-related genes, activation of PPAR γ also upregulates anti-inflammatory genes and modulates lipid metabolism, both of which may contribute to the protective effects of AFGE against ALD.

From a translational perspective, the pharmacokinetics (PK) and long-term safety of AFGE merit consideration. After oral administration, alliin is rapidly absorbed and eliminated, whereas allicin and other organosulfur compounds show later peak times and prolonged blood exposure, with extensive metabolism to polar sulfur metabolites in the intestinal tract and liver^[17, 45]. Further studies are needed to quantify plasma and hepatic exposure to AFGE and its metabolites, and to correlate these pharmacokinetic parameters with the extent of PPAR γ activation. Regarding long-term safety, previous rodent and clinical studies have generally shown that processed garlic preparations are well tolerated during prolonged administration, with adverse effects mostly limited to mild gastrointestinal symptoms^[77-78]. Nonetheless, AFGE-specific chronic toxicity studies are required to define its long-term safety.

Although our data demonstrate AFGE activated hepatic PPAR γ and up-regulated PPAR γ target genes in alcohol-treated mice, and pharmacological inhibition of PPAR γ with T0070907 attenuated the cytoprotective effects of AFGE *in vitro*, these findings do not conclusively establish the *in vivo* necessity of PPAR γ . Further studies employing hepatocyte-specific PPAR γ knockout mice or *in vivo* pharmacological inhibition are warranted.

5. Conclusions

In summary, this study demonstrates that inactivation of alliinase effectively prevents the conversion of alliin to allicin, resulting in the preparation of AFGE. AFGE activates PPAR γ , leading to the transcriptional

upregulation of antioxidant-related genes, including *Gpx3*, *Gsto2*, and *Gsta1*, thereby mitigating oxidative stress and alleviating alcohol-induced liver injury. These findings provide strong evidence supporting the potential of AFGE as a functional food for the prevention and management of ALD.

Declaration of interests

Hao Wang is an editorial board member for Food Science and Human Wellness and was not involved in the editorial review or the decision to publish this article. The authors have no conflicts of interest to this work.

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