Lipids in Health and Disease

RESEARCH

Open Access

Green tea epigallocatechin gallate attenuate metabolic dysfunction-associated steatotic liver disease by regulation of pyroptosis

Jie Zhang[1†], Shuangshuang Wang[1†], Ting Zhang[1], Minghui Zi[1], Shuxiang Wang[1] and Qiao Zhang[1*]

Abstract

Background Metabolic dysfunction-associated steatotic liver disease (MASLD) has become the most common chronic liver disease, with other fat-liver diseases potentially progressing to cirrhosis and hepatocellular carcinoma. Our study aimed to alleviate MASLD by EGCG inhibiting oxidative stress-mediated pyroptosis in zebrafish.

Methods The one month old wild-type zebrafh larval (50 per group) and three months old adult male zebrafish (15 per group) were treated with high-fat diet (HFD) feeding (powdered egg yolk) following by treatment with 25 μM EGCG for 15 days. Indicators related to liver damage, oxidative stress, inflammation, pyroptosis and aging were assessed using Oil Red O staining, H&E staining, commercial assay kits, enzyme-linked immunosorbent assay (ELISA) kits, and Western blot analysis.

Results The results suggest that EGCG significantly reduced fatness, severe lipid deposition, triglyceride (TG), total cholesterol (TC) and free fatty acid (FFA) levels ($p < 0.05$). EGCG markedly reduced serum ALT, AST and ameliorated liver injury in zebrafish ($p < 0.05$). EGCG also showed an antioxidant effect by reducing reactive oxygen species (ROS) production malondialdehyde (MDA) and increasing superoxide dismutase (SOD) levels ($p < 0.05$). Moreover, EGCG obviously down-regulated the pro-inflammatory factors like tumor necrosis factor-a (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6), and interleukin-18 (IL-18) levels ($p < 0.05$). EGCG indicated a significant upregulation involved in pyroptosis pathway, such as nuclear factor erythroid 2-related factor 2 (NRF2) and downregulated the expressions of nuclear factor-κB (NF-κB), NOD-like receptor thermal protein domain associated protein 3 (NLRP3), apoptosis-associated speck-like protein containing a CARD (ASC), cysteinyl aspartate specific proteinase-1 (caspase-1), as well as gasdermin D (GSDMD) ($p < 0.01$). Moreover, EGCG significantly improved aging-related markers induced by a HFD, including the level of senescence-associated β-Galactosidase (SA β-Gal) and expression of p53, p16, and p21 ($p < 0.05$), while ameliorating liver function in zebrafish.

Conclusions These results suggest that EGCG may attenuate MASLD in larval and adult zebrafish induced by 15 consecutive days HFD, which is potentially mediated by modulating the Nrf2/NF-κB/NLRP3 signaling pathway which relieve pyroptosis.

Keywords EGCG, High-fat diet, MASLD, Pyroptosis, Zebrafish

Introduction

Metabolic dysfunction-associated steatotic liver disease (MASLD) is the most common cause of chronic liver disease and the primary cause of liver-related morbidity and mortality [1]. MASLD formerly termed non-alcoholic fatty liver disease (NAFLD), represents a leading cause of

†Jie Zhang and Shuangshuang Wang contributed equally to this work.

*Correspondence:
Qiao Zhang
qq949495028@163.com
[1] School of Public Health, Kunming Medical University, Kunming 650500, Yunnan, China

chronic liver injury globally, affecting approximately 30% of adults and increasingly prevalent in pediatric populations due to rising obesity and metabolic syndrome rates [2]. MASLD is characterized by hepatic lipid accumulation (steatosis) progressing to inflammation (metabolic dysfunction-associated steatohepatitis, MASH), fibrosis, and hepatocellular carcinoma. MASLD pathogenesis involves intricate interplay among insulin resistance, lipotoxicity, oxidative stress, and dysregulated immune responses (e.g., NLRP3 inflammasome activation) [3]. The complexity of MASLD underscores the need for multifaceted therapeutic strategies to prevent disease progression.

Among the natural compounds under investigation for their therapeutic potential, epigallocatechin gallate (EGCG), which is abundant in green tea, has garnered considerable attention owing to its antioxidant, anti-inflammatory, and lipid-modulating properties, making it a promising candidate for managing metabolic and liver diseases [4]. Prior studies have demonstrated that EGCG can ameliorate high-fat diet-induced lipid accumulation and inflammation in liver cells such as HepG2 hepatocytes [5]. Despite these encouraging findings, the exact mechanisms through which EGCG exerts its beneficial effects, particularly in the context of pyroptosis, remain unclear.

Pyroptosis is a form of programmed cell death characterized by the activation of inflammasomes such as NLRP3, leading to the release of pro-inflammatory cytokines such as IL-1β and IL-18, which contribute to liver damage and disease progression [6, 7]. In the context of MASLD, pyroptosis has been implicated as a key player in the inflammatory response, exacerbating liver injury and fibrosis [8]. While EGCG has been shown to influence lipid metabolism and inflammation, its role in modulating pyroptosis and its associated inflammatory pathways in the context of MASLD remains unclear.

By activating the Nrf2 and inhibiting the TLR4/NF-κB pathway, EGCG attenuates hepatic steatosis and inflammatory responses in rats and mice [9, 10]. Furthermore, EGCG protects the liver from lipotoxicity by inhibiting mitochondrial ROS-mediated iron metabolism, a key process in the deterioration of NAFLD [11]. The prior investigations have predominantly elucidated the antioxidative and anti-inflammation effects of EGCG mediated by Nrf2, NF-κB. The precise mechanisms through which EGCG ameliorates MASLD via pyroptosis regulation remain to be fully elucidated, representing a critical gap in understanding its therapeutic potential targeting gasdermin D (GSDMD)-mediated inflammatory cascades.

This study aimed to investigate whether EGCG could alleviate MASLD by inhibiting pyroptosis in zebrafish, thereby offering a therapeutic targeting strategy for treating MASLD. The zebrafish model demonstrates significant advantages for studying MASLD, including high genetic homology (sharing ~70% of human NAFLD-associated genes), rapid disease modeling capabilities (steatosis induction within 7–10 days via high-fat diet or genetic manipulation), conserved liver pathology (zebrafish develop key MASLD features—mimicking human disease progression), cost-effective maintenance (low husbandry costs and short reproductive cycles) [12]. Therefore, zebrafish provide an ideal model for studying metabolic diseases and testing potential therapeutics. In this study, we examined whether EGCG treatment could reduce fat accumulation, oxidative stress, and inflammation in zebrafish fed a high-fat diet, while also investigating the molecular mechanisms involved. Specifically, we explored the regulation of pyroptosis through key signaling pathways, such as Nrf2, NF-κB, NLRP3, GSDMD, p53, p16, and p21. By elucidating the molecular pathways through which EGCG affects pyroptosis and inflammation, this study aims to offer valuable insights into its potential as a therapeutic agent for MASLD.

Materials and methods

Zebrafish maintenance and treatment

Wild-type zebrafish (AB strain) were obtained from the National Zebrafish Resource Center (Wuhan, China). Zebrafish were maintained under standard laboratory conditions with a 14:10 light–dark cycle at 28 °C in a recirculating water system [13, 14]. The one month old wild-type zebrafh larval (50 per group) and three months old adult male zebrafish (15 per group) were randomly divided into a control group, high-fat diet (HFD) group, and HFD + EGCG group. In the HFD + EGCG group, zebrafish were exposed to HFD and EGCG simultaneously for the entire experimental duration without a preceding HFD-only period. All groups were maintained under identical environmental conditions throughout the study. The normal diet (ND) group of zebrafish was fed 30 mg of freshly hatched live artemia (Huizhong Fisheries Co., Ltd., Shandong, China) per fish daily. The high-fat diet was prepared by mixing cholesterol and powdered egg yolk at a 1:9 ratio. The mixture was dissolved in anhydrous ethanol and heated to 80 °C to evaporate the ethanol. The HFD group was fed 30 mg of artemia and 70 mg of HFD per fish daily. EGCG was purchased from Sigma-Aldrich (St. Louis, MO, USA). Larval zebrafish (1 month old) and adult zebrafish (3 months old) were exposed to a high-fat diet and treated with 25 μM EGCG for 15 days. The study approved by the Experimental Animal Ethics Committee of Hunter Biotechnology, Inc. (IACUC-2024–10402-01).

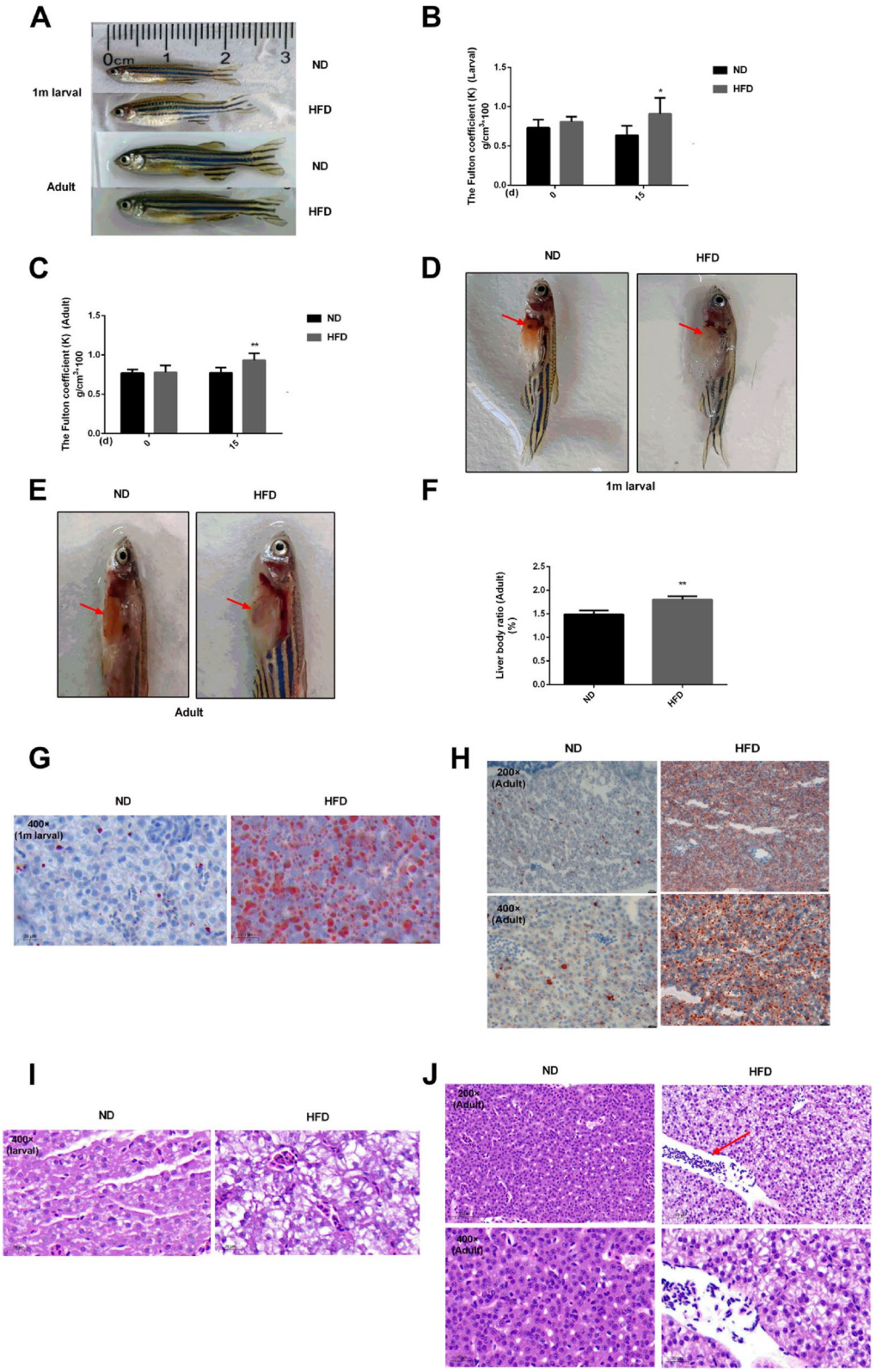


Fig. 1 Zebrafish body size and liver phenotype. The high -fat diet (HFD) group showed a significantly obese body shape (**A**), K value (**B**-**C**), liver phenotype (**D**-**E**), liver body ratio (**F**), Oil Red O staining (**G**-**H**), hematoxylin and eosin staining (**I**-**J**) compared to the normal diet (ND) group. Data are presented as means ± SD, $^{**}p < 0.01$, $^{***}p < 0.001$

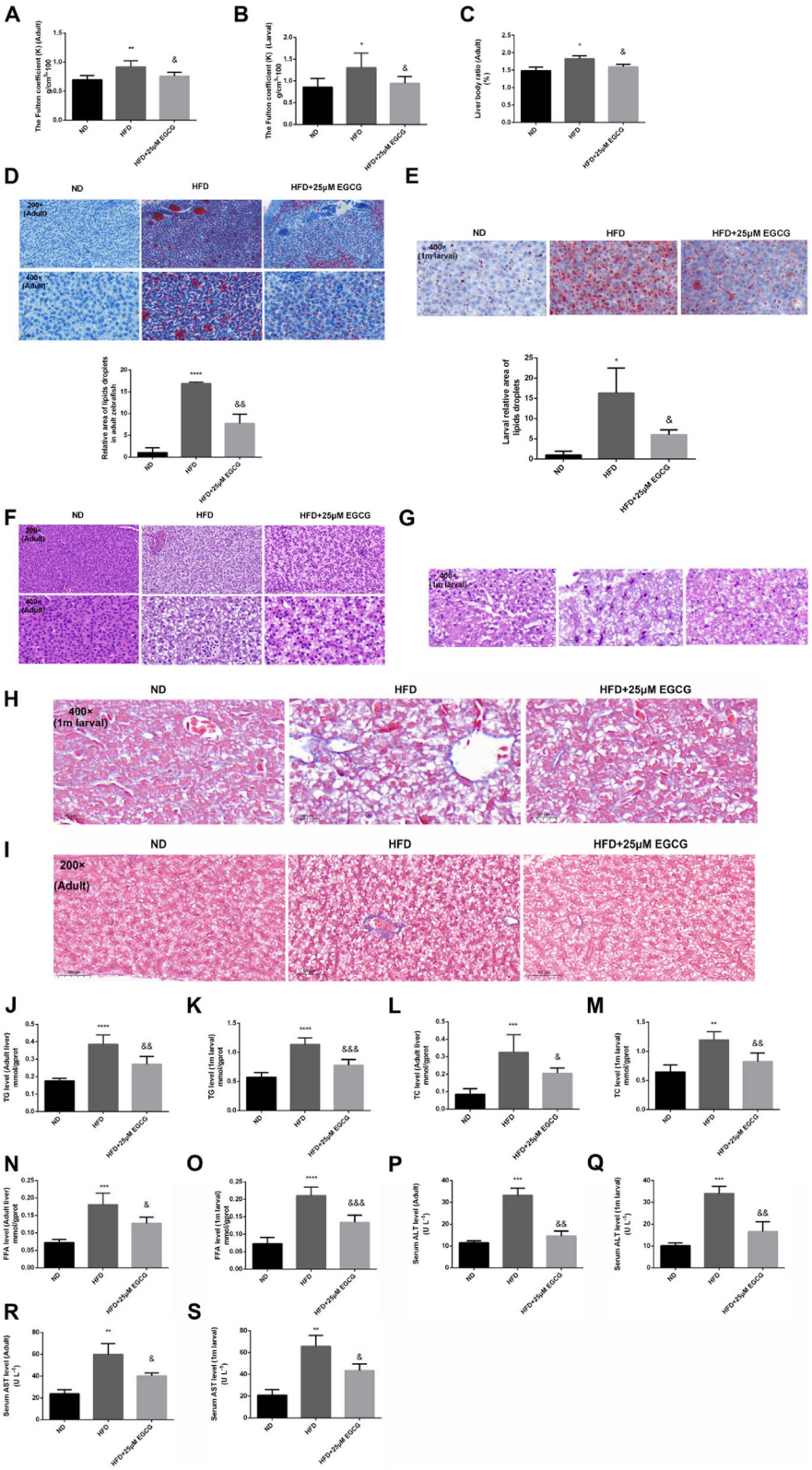


Fig. 2 (See legend on previous page.)

(See figure on next page.)

Fig. 2 EGCG mitigates hepatic lipid accumulation and pathological alterations induced by a high-fat diet in zebrafish. Effects of EGCG on K value (**A**-**B**), liver body ratio (**C**), Oil Red O staining (**D**-**E**), hematoxylin and eosin staining (**F**-**G**), Masson staining (**H**-**I**), triglyceride (**J**-**K**), total cholesterol (**L**-**M**), free fatty acid (**N**–**O**), alanine transaminase (**P**-**Q**), aspartate transferase (**R**-**S**) in adult and larval zebrafish induced by HFD. Data are presented as means ± SD, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$, compared to ND. & $p < 0.05$, && $p < 0.01$, &&& $p < 0.001$, &&&& $p < 0.0001$, compared to HFD

Fulton's body condition factor K

The Fulton coefficient (K) is an index of body condition that relates length and weight, indicates relative fatness and was designed to determine growth in fish. Zebrafish were anesthetized in fish culture water containing 0.05% tricaine. After anesthesia, the surface of the fish was carefully dried using absorbent paper. The weight of each fish was promptly measured using a balance and the length was recorded from the mouth to the tail using a ruler. The K factor of the zebrafish was calculated using the formula $K = (\text{weight (g)}/\text{length(cm)}^3) \times 100$ [15].

Liver Body Ratio (LBR) measurement

Following treatment, zebrafish were anesthetized and euthanized. The livers were carefully dissected under a stereomicroscope to avoid damage to surrounding tissues. Both liver and total body weights were measured immediately using a high-precision electronic balance.

Oil Red O staining

Zebrafish were fixed in 4% neutral formaldehyde for 30 min at room temperature and then thoroughly washed with phosphate-buffered saline (PBS) for five minutes to remove the residual fixative. Following fixation, samples were stained with 0.5% Oil Red O solution (Solarbio Science & Technology Co.,Ltd. Beijing, China) for 1 h at 37 °C. Excess stain was removed by rinsing the zebrafish with 60% isopropyl alcohol until the background appeared clear [13].

Hematoxylin and eosin (H&E) staining

Hematoxylin and eosin (H&E) staining was performed to evaluate and quantify lesion areas in liver tissue sections. The sections were first stained with hematoxylin for 3 min and then rinsed in distilled water for 10 min to remove the excess stain. Subsequently, the sections were stained with eosin for 3 min. Following staining, the sections were dehydrated through an ascending ethanol series (70%, 90%, 100%), cleared in xylene, and observed under a microscope for histological analysis [16].

Masson staining

At the end of zebrafish euthanasia, liver tissues were collected, fixed in 4% neutral buffered formalin, decalcified if necessary, and processed for paraffin embedding. Sections of 4–6 μm thickness were prepared using a microtome. Masson staining was performed following the protocol provided with the staining kit to evaluate collagen fiber deposition in the liver. Collagen fibers appeared blue under microscopic examination, indicating fibrosis [17].

Biochemical analysis

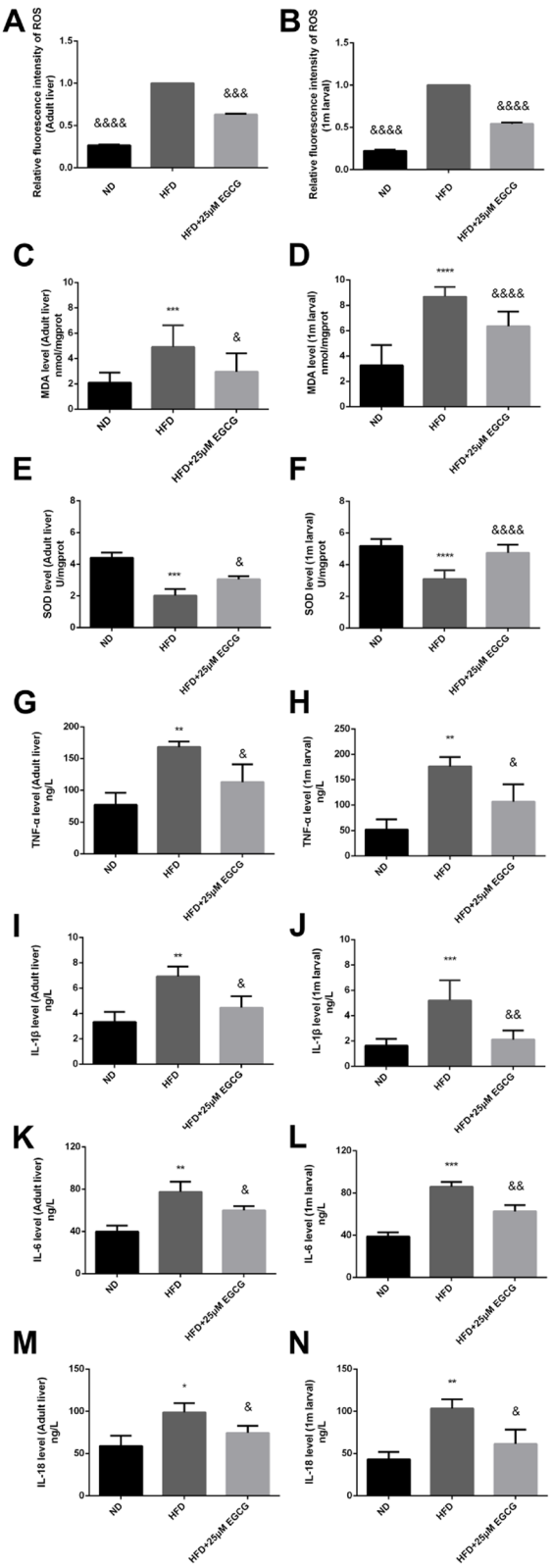
Zebrafish tissues were homogenized in lysis buffer, and the lysates were centrifuged to obtain the supernatants. Total cholesterol (TC), triglyceride (TG), free fatty acid (FFA), malondialdehyde (MDA) levels, and superoxide dismutase (SOD) activity were measured using commercial kits (Jiancheng, Nanjing, China). Inflammatory cytokines, including IL-1β, IL-18, TNF-α, and IL-6, were quantified in zebrafish using enzyme-linked immunosorbent assay (ELISA) kits (Jiancheng, Nanjing, China), following the protocols provided by the manufacturer. Following centrifugation of the blood collected from the tail of the fish, serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined using commercially available kits (Jiancheng, Nanjing, China).

Senescence-associated β-galactosidase staining

Senescence in larval and adult zebrafish was evaluated using β-galactosidase staining (Jiancheng, Nanjing, China).

Western blot

The fish were dried, placed in a grinding bowl with liquid nitrogen, and homogenized. Proteins were extracted using a frozen RIPA lysis buffer supplemented with phosphatase and protease inhibitor cocktails. Protein concentrations were quantified using a BCA protein assay. The samples were subjected to immunoblotting for protein detection. β-actin was used as an internal loading control to ensure equal protein loading and normalization across samples. Primary antibodies were incubated overnight at 4 °C, followed by incubation with secondary antibodies the following day for chemiluminescence-based



◀ **Fig. 3** EGCG attenuate high-fat-induced oxidative stress and inflammation in zebrafish. Effects of EGCG on reactive oxygen species (**A**-**B**), malondialdehyde (**C**-**D**), superoxide dismutase (**E**–**F**), tumor necrosis factor-α (**G**-**H**), interleukin-1β (**I**-**J**), interleukin-6 (**K**-**L**), and interleukin-18 (3 M-3 N) levels in HFD induced larval and adult zebrafish. Data are presented as means ± SD, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$, compared to ND. & $p < 0.05$, && $p < 0.01$, &&& $p < 0.001$, &&&& $P < 0.0001$, compared to HFD

detection. The following antibodies were used: TNF-α (Affinity Biosciences, Jiangsu, China, MW17), NLRP3 (Affinity Biosciences, Jiangsu, China, MW80), Nrf2 (HUABIO, Wuhan, China, MW100), p53 (ABclonal Technology, Wuhan, China, MW45), NF-κB (HUABIO, Wuhan, China, MW65), IL-1β (Santa Cruz Biotechnology, MW17), IL-6 (ABclonal Technology, Wuhan, China, MW24), IL-18 (Affinity Biosciences, Jiangsu, China, MW22), β-actin (ABclonal Technology, Wuhan, China, MW42), caspase-1 (Affinity Biosciences, Jiangsu, China, MW45), caspase-1 p20 (ABclonal Technology, Wuhan, China, MW29), GSDMD (ABclonal Technology, Wuhan, China, MW39), p16 (Santa Cruz Biotechnology, MW16), p21 (Santa Cruz Biotechnology, MW21) and ASC (Proteintech Biotechnology, Wuhan, China, MW25). The following day, an alkaline phosphatase-conjugated anti-rabbit or anti-mouse secondary antibody (KPL, Los Angeles, CA, USA) was applied for 1 h at 30 °C. Protein signals were detected using the enhanced chemiluminescence (ECL) method [18].

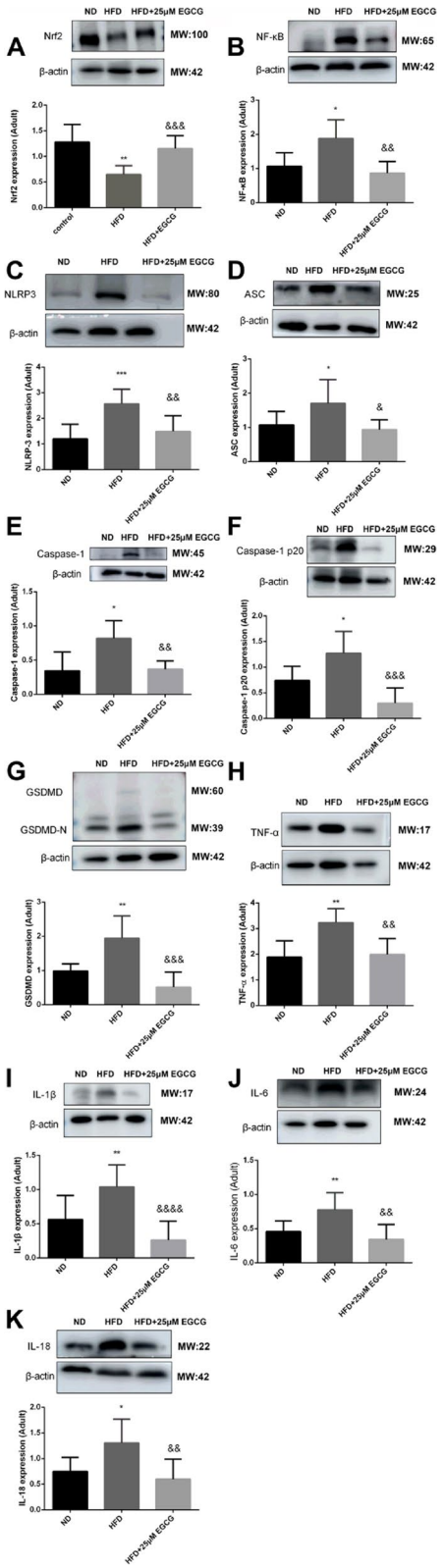
Statistical analysis

Statistical analyses were performed using SPSS software version 18.0, and graphs were generated using GraphPad Prism 8.0. Data are presented as mean ± standard deviation (SD). Prior to statistical testing, the normality of data distribution was assessed using the Shapiro–Wilk test. For data that conformed to a normal distribution, one-way ANOVA was conducted, followed by Dunnett's post hoc test for pairwise comparisons. A p-value < 0.05 was considered statistically significant.

Results

Establishment of a zebrafish model for MASLD

To establish the zebrafish model of MASLD, larval (1 m) and adult zebrafish (male) were fed Artemia mixed with or without egg yolk powder for 15 days, and MASLD was characterized by observing the accumulation of lipids in the liver. At the end of the feeding assay, zebrafish in the HFD group exhibited the obesity phenotype (Fig. 1A) and a significant increase in K value (Fig. 1B-C, $p < 0.05$), indicating marked obesity in the HFD group compared with the ND group. Upon exposing the abdominal cavity of zebrafish, the livers of the HFD group (Fig. 1D-E, right)



◀ **Fig. 4** Effects of EGCG on key proteins associated with pyroptosis to alleviate MASLD in adult zebrafish. The nuclear factor erythroid 2-related factor 2 (**A**), nuclear factor-kappa B (**B**), NOD-like receptor family pyrin domain containing 3 (**C**), apoptosis-associated speck-like protein containing a CARD (**D**), cysteinyl aspartate specific proteinase-1 (**E**), cysteinyl aspartate specific proteinase-1 p20 (**F**), gasdermin D (**G**), tumor necrosis factor-α (H), interleukin-1β (I), interleukin-6 (J) and interleukin-18 (**K**) protein levels were determined by western blot method. Data are presented as means ± SD, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$, compared to ND. &$p < 0.05$, &&$p < 0.01$, &&&$p < 0.001$, &&&&$p < 0.0001$, compared to HFD

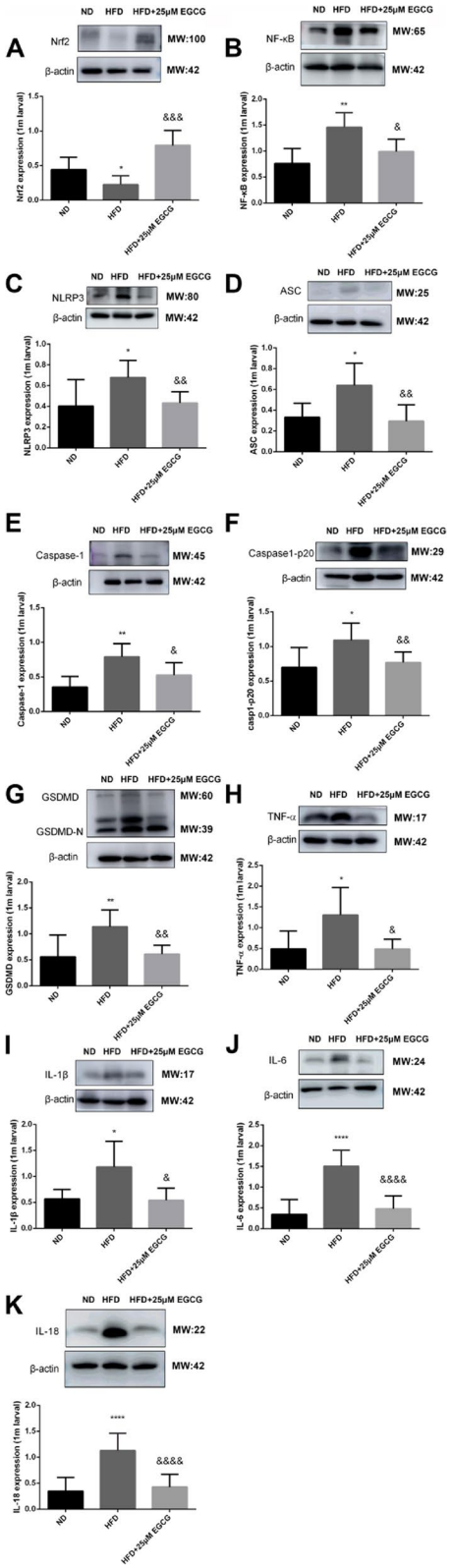
exhibited a noticeably more pronounced yellow coloration than those of the ND group (orange red, Fig. 1D-E, left). The liver body ratio (LBR) was also significantly elevated in the adult zebrafish HFD group (1.80 ± 0.04%) compared with that in the ND group (1.56 ± 0.03%, $p < 0.01$), reflecting hepatic fat accumulation (Fig. 1F). Oil Red O staining revealed substantial lipid droplet accumulation in the liver tissues of the HFD group, as evidenced by prominent red staining (Fig. 1G-H). H&E staining revealed that the livers of the HFD group exhibited inflammatory lesions (indicated by red arrows, representing the aggregation of numerous nuclei), ballooning degeneration (characterized by cytoplasmic vacuolation, with nuclei either centrally suspended or displaced to the periphery), and extensive steatotic areas, reflecting the progression of MASLD (Fig. 1I-J).

Protective effects of EGCG against hepatic steatosis and pathological changes induced by a high-fat diet in zebrafish

To investigate the effects of EGCG on hepatic steatosis and liver lipid metabolism dysfunction, we assessed related indicators in HFD-fed zebrafish. EGCG reduced K value ($p < 0.05$) and LBR ($p < 0.05$) in HFD-fed zebrafish, indicating decreased lipid deposition (Fig. 2A-C). Oil Red O and H&E staining confirmed hepatic steatosis in the HFD group, which was markedly alleviated by EGCG treatment (Fig. 2D-G). Masson staining revealed fibrosis development in larvae (Fig. 2H) but not in adult zebrafish (Fig. 2I) after 15 days of HFD exposure. Additionally, EGCG significantly lowered HFD-induced elevations in TG ($p < 0.01$), TC($p < 0.05$), FFA ($p < 0.05$) and serum ALT ($p < 0.01$) and AST ($p < 0.05$) levels in both larvae and adults (Fig. 2J-S). These results suggest that EGCG protects against lipid accumulation, liver injury, and early fibrosis by regulating lipid metabolism in zebrafish.

EGCG alleviate high-fat-induced oxidative stress and inflammation in zebrafish

EGCG treatment significantly reduced oxidative stress markers compared to the HFD group (Fig. 3A-D, $p < 0.05$) and enhanced antioxidant enzyme activity, particularly



◀ **Fig. 5** EGCG influences crucial proteins involved in pyroptosis to reduce MASLD in larval. The nuclear factor erythroid 2-related factor 2 (**A**), nuclear factor-kappa B (**B**), NOD-like receptor family pyrin domain containing 3 (**C**), apoptosis-associated speck-like protein containing a CARD (**D**), cysteinyl aspartate specific proteinase-1 (**E**), cysteinyl aspartate specific proteinase-1 p20 (**F**), gasdermin D (**G**), tumor necrosis factor-α (**H**), interleukin-1β (**I**), interleukin-6 (**J**) and interleukin-18 (**K**) protein levels were determined by western blot method. Data are presented as means ± SD, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$, compared to ND. & $p < 0.05$, && $p < 0.01$, &&& $p < 0.001$, &&&& $p < 0.0001$, compared to HFD

SOD (Fig. 3E-F, $p < 0.05$), thereby restoring the balance between oxidative damage and cellular defense mechanisms. Moreover, EGCG downregulated the HFD-induced elevations of pro-inflammatory cytokines, including TNF-α (Fig. 3G-H, $p < 0.05$), IL-1β (Fig. 3I-J, $p < 0.05$), IL-6 (Fig. 3K-L, $p < 0.05$), and IL-18 (Fig. 3M-N, $p < 0.05$), which are known to exacerbate liver injury and promote MASLD progression. These results suggest that EGCG's protective effects are partially mediated through the suppression of pyroptosis-associated inflammatory responses in both larval and adult zebrafish.

EGCG modulate critical proteins involved in pyroptosis to mitigate MASLD in high-fat-induced zebrafish

To investigate the effects and mechanisms by which EGCG suppresses HFD-induced pyroptosis and prevents MASLD, we focused on key pathways including Nrf2, NF-κB, and NLRP3. EGCG treatment significantly upregulated Nrf2 expression (Fig. 4A) while downregulating pyroptosis-associated proteins, including NF-κB (Fig. 4B), NLRP3 (Fig. 4C), ASC (Fig. 4D), caspase-1 (Fig. 4E), caspase-1 p20 (Fig. 4F), and GSDMD (Fig. 4G). Additionally, EGCG markedly reduced the expression of pyroptosis-related inflammatory cytokines, such as TNF-α (Fig. 4H), IL-1β (Fig. 4I), IL-6 (Fig. 4J), and IL-18 (Fig. 4K), in adult zebrafish. Similar improvements were observed in larval zebrafish, where EGCG significantly alleviated HFD-induced elevations of pyroptosis markers and inflammatory factors (Fig. 5A-K).

EGCG regulates key indicators associated with aging in high-fat-induced zebrafish

EGCG effectively modulated aging-related biomarkers in zebrafish subjected to HFD. HFD exposure accelerated aging phenotypes, characterized by increased oxidative stress, inflammation, senescence-associated β-galactosidase (SA-β-gal) activity, and upregulation of key senescence markers. EGCG treatment significantly reduced SA-β-gal activity (Fig. 6A-B) and downregulated the expression of p53 (Fig. 6C-D), p16 (Fig. 6E-F), and p21 (Fig. 6G-H), key regulators of cellular senescence. These findings suggest that EGCG may delay the onset

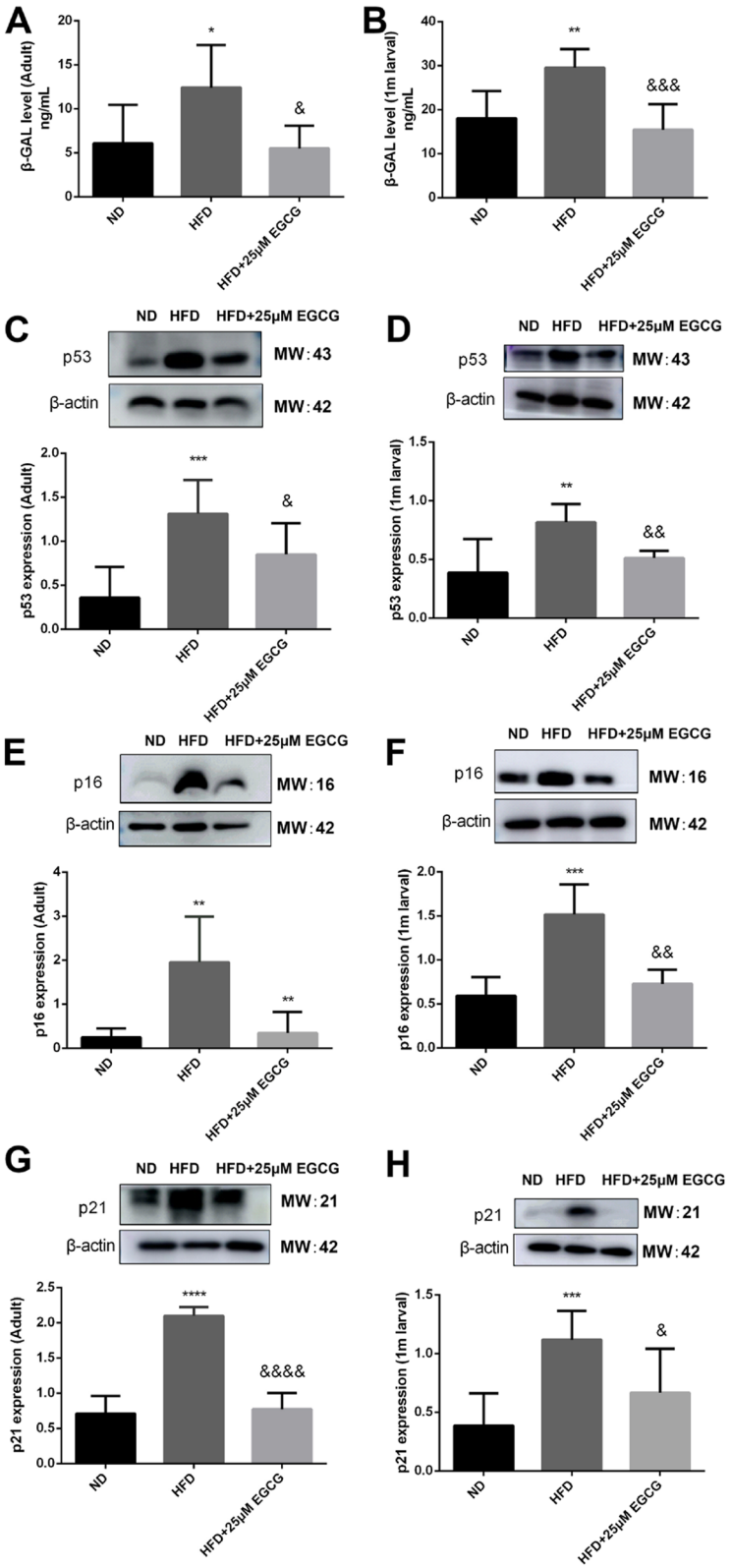


Fig. 6 EGCG modulates essential aging-related factors in HFD-induced zebrafish. The senescence-associated β-Galactosidase levels (**A**-**B**), and p53 (**C**-**D**), p16 (**E**–**F**) and p21 (**G**-**H**) protein expressions. Data are presented as means ± SD, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$, compared to ND. & $p < 0.05$, && $p < 0.01$, &&& $p < 0.001$, &&&& $p < 0.0001$, compared to HFD

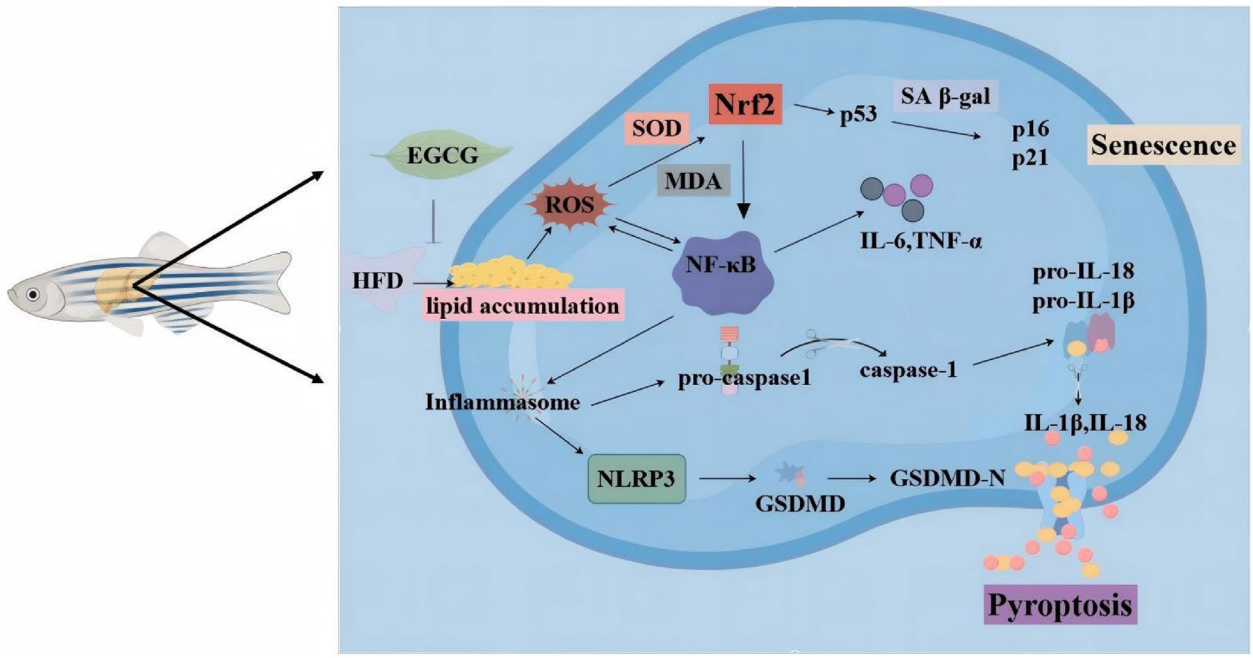


Fig. 7 Nrf2 signaling pathway of EGCG in the treatment of MASLD on zebrafish

of cellular aging and protect against age-related liver dysfunction in HFD-fed zebrafish.

Discussion

Metabolic dysfunction-associated steatotic liver disease (MASLD) has reached epidemic proportions globally, with an estimated global prevalence of 30–35% in adults, translating to over 1.5 billion affected individuals. Regional variations are significant, with higher rates in South America (40–45%) and the Middle East (35–40%) compared to North America (30–32%) and Europe (25–28%), driven by obesity and metabolic syndrome disparities [19]. Notably, 70–80% of individuals with type 2 diabetes and 90% of those with severe obesity (BMI ≥ 40 kg/m^2) exhibit MASLD, underscoring its role as a multisystem metabolic disorder. Projections suggest MASLD-associated cirrhosis cases will surge by 168% by 2030, with hepatocellular carcinoma incidence rising commensurately [20]. These trends highlight an urgent need for novel therapeutic strategies targeting the complex molecular mechanisms underlying the disease.

An effective approach to mitigating symptoms associated with liver diseases, particularly MASLD, using anti-inflammatory agents. Key strategies to retard or minimize inflammation-driven MASLD focus on combating pyroptosis triggered by a HFD. Green tea and its primary component, EGCG, are known for their antioxidant, anti-inflammatory, and anti-aging properties [21]. Studies have confirmed that EGCG exhibits significant anti-inflammatory effects by inhibiting the production of pro-inflammatory factors induced by HFD [11]. Furthermore, EGCG improves NAFLD primarily by regulating molecular mechanisms, such as autophagy, mitochondrial function, and oxidative stress. Its effects are predominantly mediated through signaling pathways such as PI3 K/Akt/mTOR, AMPK, and NF-κB [22]. However, the mechanisms by which EGCG protects zebrafish with HFD-induced MASLD through inhibition of pyroptosis pathways remain unclear.

Zebrafish has emerged as a prominent model vertebrate, with approximately 70% of human genes having at least one zebrafish ortholog. The National Institutes of Health (NIH) ranks zebrafish as the third most widely used vertebrate model organism, after mice and rats. It has been reported that EGCG effectively ameliorated NAFLD phenotypes and metabolic disorders in rats fed a HFD [9]. Our results also demonstrated that EGCG significantly reduced fat accumulation and lipid droplet formation in zebrafish liver. TG, TC and FFA levels were lowered in the EGCG-treated group compared to the high-fat diet group. This highlights the ability of EGCG to restore lipid homeostasis and prevent excessive hepatic lipid accumulation, which is a hallmark of MASLD.

Oxidative stress and inflammation are critical drivers of MASLD progression, contributing to liver damage and metabolic dysfunction [23]. In our study, EGCG significantly decreased ROS and MDA levels while enhancing

the activity of SOD, an essential antioxidant enzyme. These findings suggest that EGCG restores the redox balance and mitigates oxidative damage in the liver. Furthermore, EGCG downregulated key pro-inflammatory cytokines, including TNF-α, IL-1β, IL-6, and IL-18, which are associated with the exacerbation of lipid accumulation, hepatocyte injury, and fibrogenesis in MASLD. These findings highlight the dual antioxidant and anti-inflammatory effects of EGCG as a comprehensive protective strategy.

Pyroptosis, a form of programmed inflammatory cell death, has been implicated in the pathogenesis of MASLD [24]. It is characterized by rapid cytoplasmic disintegration and release of intracellular pro-inflammatory mediators. These factors contribute significantly to local inflammation and exacerbate pathological processes associated with MASLD [25]. Nrf2 acts as a master regulator of antioxidant defense by promoting the transcription of cytoprotective genes, thereby attenuating oxidative stress, suppressing inflammation and pyroptosis [26]. Impaired activation of Nrf2 leads to excessive accumulation of ROS, which in turn activates NF-κB, a key transcription factor orchestrating the expression of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6 [27–29]. Persistent NF-κB activation further enhances the priming and activation of the NLRP3 inflammasome, culminating in caspase-1 activation, GSDMD cleavage, and pyroptotic cell death. This cascade promotes hepatocyte injury, lipid accumulation, and fibrosis, contributing to the exacerbation of MASLD [30–32]. Our study corroborates these findings: EGCG treatment activated Nrf2 signaling, inhibited NF-κB activation, suppressed NLRP3 inflammasome assembly (including adaptor protein ASC and caspase-1 activation), and reduced cleavage of GSDMD, a key executor of pyroptosis. This inhibition ultimately curtailed the secretion of pyroptosis-associated inflammatory cytokines (TNF-α, IL-1β, IL-6, and IL-18), illustrating the mechanistic basis for EGCG's hepatoprotective effects. It is important to note that the observed attenuation of hepatic injury may not be solely attributable to direct inhibition of pyroptosis. EGCG's broad anti-inflammatory effects could indirectly suppress pyroptosis by reducing upstream inflammatory signaling and oxidative stress. Thus, distinguishing between direct and secondary effects remains a challenge.

In addition, we found that EGCG ameliorated hepatic aging markers, such as β-galactosidase activity and expression of p53, p16, and p21, while improving liver function. These indicators were selected because cellular senescence is increasingly recognized as a contributor to MASLD-related liver dysfunction and fibrosis. The p53/p21, p16 pathways orchestrate the senescence response, promoting chronic inflammation (senescence-associated secretory phenotype, SASP) and tissue deterioration. A study showed that EGCG has a protective effect against cellular senescence and associated liver dysfunction by reducing oxidative stress and inflammation [33]. Thus, their modulation by EGCG suggests a dual action against both pyroptosis-driven and senescence-driven hepatic injury. These results position EGCG as a promising candidate for the integrative management of MASLD by targeting oxidative stress, inflammation, pyroptosis, and cellular senescence (Fig. 7). Future studies should explore the upstream regulatory networks modulated by EGCG and assess its translational potential in human MASLD populations.

Strengths and limitations

This study offers novel insights into the protective role of EGCG in MASLD by demonstrating its capacity to modulate oxidative stress, inflammatory signaling, pyroptosis, and senescence pathways in a high-fat diet-induced zebrafish model. The integrated analysis of lipid metabolism, redox balance, and inflammatory and aging markers reinforces the biological plausibility of EGCG's multi-targeted effects.

Nevertheless, several limitations must be acknowledged. First, the study lacks direct mechanistic validation through genetic or pharmacological modulation of key pathways such as Nrf2, NF-κB, and NLRP3. Second, only a single EGCG dose was tested, preventing evaluation of dose-dependent effects and optimal therapeutic ranges. Third, species-specific differences in liver metabolism between zebrafish and mammals may limit the generalizability of the findings. Moreover, the preventive design and short-term intervention preclude conclusions about EGCG's therapeutic efficacy and long-term effects.

Future studies should focus on mechanistic validation, dose–response analyses, confirmation in mammalian MASLD models, and long-term efficacy and safety assessments to support the clinical translation of EGCG-based therapies.

Conclusion

In summary, this study advances our understanding of EGCG's protective mechanisms against HFD-induced MASLD, highlighting its ability to attenuate oxidative stress, inflammation, pyroptosis, and cellular senescence. Although our findings suggest that EGCG ameliorates MASLD partly through suppression of pyroptosis-related pathways, further mechanistic studies are warranted to distinguish direct effects on pyroptosis from secondary consequences of reduced inflammation. These findings provide a mechanistic basis for EGCG's multi-targeted effects and underscore the need for further preclinical and clinical studies to validate its therapeutic potential.

Abbreviations

HFD	High fat diet
TG	Ttriglyceride
TC	Total cholesterol
MASLD	Metabolic dysfunction-associated fatty liver disease
ROS	Reactive oxygen species
MDA	Malondialdehyde
SOD	Superoxide dismutase
TNF-α	Tumor necrosis factor-a
IL-1β	Interleukin (IL)-1β
IL-6	Interleukin (IL)-6
IL-18	Interleukin (IL)-18
Nrf2	Nuclear factor erythroid 2-related factor 2
NF-κB	Nuclear factor-κB
NLRP3	NOD-like receptor thermal protein domain associated protein 3
ASC	Apoptosis-associated speck-like protein containing a CARD
caspase-1	Cysteinyl aspartate specific proteinase-1
GSDMD	Gasdermin D
SA β-Gal	Senescence-associated β-Galactosidase

Acknowledgements
Not applicable.

Authors' contributions
Qiao Zhang conceived the idea and designed this study. Jie Zhang and Shuangshuang Wang performed the experiments and wrote the manuscript, prepared the figures, and were responsible for data compilation and integration. Ting Zhang, Minghui Zi, and Shuxiang Wang revised the English text. All authors contributed to discuss the results and to research directions.

Funding
This study was supported by Yunnan Fundamental Research Projects (grant NO:202201 AT070133), National Natural Science Foundation of China (NO: 82160619).

Data availability
Data is provided within the manuscript or supplementary information files.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 10 March 2025 Accepted: 7 May 2025
Published online: 17 May 2025

References

1. Chan WK, Chuah KH, Rajaram RB, Lim LL, Ratnasingam J, Vethakkan SR. Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD): a state-of-the-art review. J Obes Metab Syndr. 2023;32(3):197–213.
2. Rinella ME, Lazarus JV, Ratziu V, Francque SM, Sanyal AJ, Kanwal F, Romero D, Abdelmalek MF, Anstee QM, Arab JP, Arrese M, Bataller R, Beuers U, Boursier J, Bugianesi E, Byrne CD, Castro Narro GE, Chowdhury A, Cortez-Pinto H, Cryer DR, Cusi K, El-Kassas M, Klein S, Eskridge W, Fan J, Gawrieh S, Guy CD, Harrison SA, Kim SU, Koot BG, Korenjak M, Kowdley KV, Lacaille F, Loomba R, Mitchell-Thain R, Morgan TR, Powell EE, Roden M, Romero-Gómez M, Silva M, Singh SP, Sookoian SC, Spearman CW, Tiniakos D, Valenti L, Vos MB, Wong VW, Xanthakos S, Yilmaz Y, Younossi Z, Hobbs A, Villota-Rivas M, Newsome PN. A multisociety Delphi consensus statement on new fatty liver disease nomenclature. J Hepatol. 2023;79(6):1542–56.
3. Li Y, Yang P, Ye J, Xu Q, Wu J, Wang Y. Updated mechanisms of MASLD pathogenesis. Lipids Health Dis. 2024;23(1):117.
4. Esmaeelpanah E, Razavi BM, Hosseinzadeh H. Green tea and metabolic syndrome: a 10-year research update review. Iran J Basic Med Sci. 2021;24(9):1159–72.
5. Zhang Q, Yuan H, Zhang C, Guan Y, Wu Y, Ling F, Niu Y, Li Y. Epigallocatechin gallate improves insulin resistance in HepG2 cells through alleviating inflammation and lipotoxicity. Diabetes Res Clin Pract. 2018;142:363–73.
6. Knorr J, Wree A, Feldstein AE. Pyroptosis in steatohepatitis and liver diseases. J Mol Biol. 2022;434(4):167271.
7. Li R, Xue W, Wei H, Fan Q, Li X, Qiu Y, Cui D. Research progress of pyroptosis in fatty liver disease. Int J Mol Sci. 2023;24(17):13065.
8. Vasudevan SO, Behl B, Rathinam VA. Pyroptosis-induced inflammation and tissue damage. Semin Immunol. 2023;69:101781.
9. Zuo G, Chen M, Zuo Y, Liu F, Yang Y, Li J, Zhou X, Li M, Huang JA, Liu Z, Lin Y. Tea polyphenol epigallocatechin gallate protects against nonalcoholic fatty liver disease and associated endotoxemia in rats via modulating gut microbiota dysbiosis and alleviating intestinal barrier dysfunction and related inflammation. J Agric Food Chem. 2024.
10. Hou H, Yang W, Bao S, Cao Y. Epigallocatechin gallate suppresses inflammatory responses by inhibiting toll-like receptor 4 signaling and alleviates insulin resistance in the livers of high-fat-diet rats. J Oleo Sci. 2020;69(5):479–86.
11. Ding SB, Chu XL, Jin YX, Jiang JJ, Zhao X, Yu M. Epigallocatechin gallate alleviates high-fat diet-induced hepatic lipotoxicity by targeting mitochondrial ROS-mediated ferroptosis. Front Pharmacol. 2023;14:1148814.
12. Tonon F, Grassi G. Zebrafish as an Experimental Model for Human Disease. Int J Mol Sci. 2023;24(10):8771.
13. Li L, Xiong Y, Cao W, Chen Z, He L, Tong M, Zhang L, Wu M. Epidermal growth factor receptor promotes high-fructose nonalcoholic fatty liver disease by inducing mitochondrial fission in zebrafish. Biochem Biophys Res Commun. 2023;652:112–20.
14. Liu Y, Zhu D, Liu J, Sun X, Gao F, Duan H, Dong L, Wang X, Wu C. Pediococcus pentosaceus PR-1 modulates high-fat-died-induced alterations in gut microbiota, inflammation, and lipid metabolism in zebrafish. Front Nutr. 2023;10:1087703.
15. Teame T, Zhang Z, Ran C, Zhang H, Yang Y, Ding Q, Xie M, Gao C, Ye Y, Duan M, Zhou Z. The use of zebrafish (Danio rerio) as biomedical models. Anim Front. 2019;9(3):68–77.
16. Li Z, Ni M, Yu H, Wang L, Zhou X, Chen T, Liu G, Gao Y. Gut microbiota and liver fibrosis: one potential biomarker for predicting liver fibrosis. Biomed Res Int. 2020;2020:3905130.
17. Ye S, Zhao T, Zhang W, Tang Z, Gao C, Ma Z, Xiong J-W, Peng J, Tan W-Q, Chen J. p53 isoform Δ113p53 promotes zebrafish heart regeneration by maintaining redox homeostasis. Cell Death Dis. 2020;11(7):568.
18. Zhang J, Xu Y, Ruan X, Zhang T, Zi M, Zhang Q. Photoprotective effects of epigallocatechin gallate on ultraviolet-induced zebrafish and human skin fibroblasts cells. Mediators Inflamm. 2024;2024:7887678.
19. Younossi ZM, Golabi P, Paik JM, Henry A, Van Dongen C, Henry L. The global epidemiology of nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH): a systematic review. Hepatology. 2023;77(4):1335–47.
20. Estes C, Anstee QM, Arias-Loste MT, Bantel H, Bellentani S, Caballeria J, Colombo M, Craxi A, Crespo J, Day CP, Eguchi Y, Geier A, Kondili LA, Kroy DC, Lazarus JV, Loomba R, Manns MP, Marchesini G, Nakajima A, Negro F, Petta S, Ratziu V, Romero-Gomez M, Sanyal A, Schattenberg JM, Tacke F, Tanaka J, Trautwein C, Wei L, Zeuzem S, Razavi H. Modeling NAFLD disease burden in China, France, Germany, Italy, Japan, Spain, United Kingdom, and United States for the period 2016–2030. J Hepatol. 2018;69(4):896–904.
21. James A, Wang K, Wang Y. Therapeutic activity of green tea epigallocatechin-3-gallate on metabolic diseases and non-alcoholic fatty liver diseases: the current updates. Nutrients. 2023;15(13):3022.
22. Tang G, Xu Y, Zhang C, Wang N, Li H, Feng Y. Green Tea and Epigallocatechin Gallate (EGCG) for the management of Nonalcoholic Fatty Liver Diseases (NAFLD): insights into the role of oxidative stress and antioxidant mechanism. Antioxidants (Basel). 2021;10(7):1076.
23. Jakubek P, Kalinowski P, Karkucinska-Wieckowska A, Kaikini A, Simões ICM, Potes Y, Kruk B, Grajkowska W, Pinton P, Milkiewicz P, Grąt M, Pronicki M, Lebiedzinska-Arciszewska M, Krawczyk M, Wieckowski MR. Oxidative stress in metabolic dysfunction-associated steatotic liver disease

(MASLD): how does the animal model resemble human disease? Faseb J. 2024;38(3):e23466.
24. Al Mamun A, Wu Y, Jia C, Munir F, Sathy KJ, Sarker T, Monalisa I, Zhou K, Xiao J. Role of pyroptosis in liver diseases. Int Immunopharmacol. 2020;84:106489.
25. Schwärzler J, Grabherr F, Grander C, Adolph TE, Tilg H. The pathophysiology of MASLD: an immunometabolic perspective. Expert Rev Clin Immunol. 2024;20(4):375–86.
26. Casper E. The crosstalk between Nrf2 and NF-κB pathways in coronary artery disease: can it be regulated by SIRT6? Life Sci. 2023;330:122007.
27. Zhang B, Yu J, Bao L, Feng D, Qin Y, Fan D, Hong X, Chen Y. Cynarin inhibits microglia-induced pyroptosis and neuroinflammation via Nrf2/ROS/NLRP3 axis after spinal cord injury. Inflamm Res. 2024;73(11):1981–94.
28. Wang M, Zhang F, Ning X, Wu C, Zhou Y, Gou Z, Fan Y, Duan R, Li Z, Shao C, Lu L. Regulating NLRP3 inflammasome-induced pyroptosis via Nrf2: TBHQ limits hyperoxia-induced lung injury in a mouse model of bronchopulmonary dysplasia. Inflammation. 2023;46(6):2386–401.
29. Liu T, Zhang L, Joo D, Sun SC. NF-κB signaling in inflammation. Signal Transduct Target Ther. 2017;2:17023.
30. Swanson KV, Deng M, Ting JP. The NLRP3 inflammasome: molecular activation and regulation to therapeutics. Nat Rev Immunol. 2019;19(8):477–89.
31. Upadhyay KK, Jadeja RN, Vyas HS, Pandya B, Joshi A, Vohra A, Thounaojam MC, Martin PM, Bartoli M, Devkar RV. Carbon monoxide releasing molecule-A1 improves nonalcoholic steatohepatitis via Nrf2 activation mediated improvement in oxidative stress and mitochondrial function. Redox Biol. 2020;28:101314.
32. Roy S, Saha P, Bose D, Trivedi A, More M, Xiao S, Diehl AM, Chatterjee S. Hepatic NLRP3-derived Hsp70 binding to TLR4 mediates MASLD to MASH Progression upon inhibition of PP2A by harmful algal bloom toxin microcystin, a second Hit. Int J Mol Sci. 2023;24(22):16354.
33. Kumar R, Sharma A, Padwad Y, Sharma R. Preadipocyte secretory factors differentially modulate murine macrophage functions during aging which are reversed by the application of phytochemical EGCG. Biogerontology. 2020;21(3):325–43.

Publisher's Note