Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2025.270

RESEARCH ARTICLE

Bruceine a Attenuates Induced Fatty Liver Disease by Inhibiting CCR1 and Activating Autophagy in C57BL/6J Mice

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ARTICLE HISTORY (25-753)

Received: August 06, 2025
Revised: September 16, 2025
Accepted: September 18, 2025
Published online: September 25, 2025

Key words:
Autophagy
BA
CCR1
Fatty liver disease
Oxidative stress

A B S T R A C T

Fatty liver disease (FLD) may eventually progress to a wide-ranging spectrum, involving steatosis, liver cirrhosis and even hepatocellular carcinoma. The study aimed to explore the activity and underlying mechanism of quassinoid compound Bruceine A (BA), which was extracted from traditional Chinese medicine *Brucea javanica (L.) Merr.*, against FLD in mice. C57BL/6 mice (n=60) were divided equally into: Control (Con), FLD, BA low-, medium-, and high-dose treatment groups. FLD was induced by receiving a high-fat diet (HFD) over a 16-week period and 1, 2 and 4 mg/kg BA was administered by intraperitoneal injection every 4 days. Our study demonstrated that BA treatment significantly ameliorated FLD symptom ($P<0.05$ vs. FLD model group), evidenced by reduced body weight, decreased serum lipid profile, elevated hepatic function and attenuated hepatic steatosis. Moreover, reduced reactive oxygen species (ROS) and malondialdehyde (MDA) overproduction were found in FLD mice with BA treatment ($P<0.05$ vs. FLD model group). More significantly, network pharmacology analysis identified that BA was likely to exert therapeutic effects on FLD by down-regulating chemokine-receptor 1 (CCR1) expression, and this finding was further validated through the following *in vivo* experiments. Mechanistically, we revealed that BA supplementation promoted autophagosome formation and regulated the expression of autophagy-related markers, including L3II/I, P62 and Beclin1 ($P<0.05$ vs. FLD model group). These findings suggested that BA-induced autophagy played a crucial role in alleviating the fatty liver condition. In conclusion, this study demonstrated that BA protected against induced FLD in mice through suppression of CCR1-dependent oxidative stress and activation of autophagic pathways, thereby providing novel therapeutic insights for the management of FLD.

To Cite This Article: Hu H, Liang S, Li Y, Liu J, Wu T and Tian Y, 2025. Bruceine A attenuates induced fatty liver disease by inhibiting CCR1 and activating autophagy in C57BL/6J mice. Pak Vet J, 45(3): 1403-1410. http://dx.doi.org/10.29261/pakvetj/2025.270

INTRODUCTION

Fatty liver disease (FLD), characterized by excessive hepatic lipid accumulation, is a major metabolic disorder affecting both humans and animals, with profound implications for public health and veterinary medicine (Polyzos *et al.,* 2019; Younossi, 2019; Gross, 2023). The symptoms of FLD range from simple steatosis to life-threatening conditions, like cirrhosis and even hepatocellular carcinoma (Estes *et al.,* 2018). The rising incidence of FLD brings a substantial burden on public health systems (Powell *et al.,* 2021). In animals, FLD may lead to metabolic derangements, reduced productivity, and increased mortality (Le-Tian *et al.,* 2020; Tufarelli *et al.,* 2024). Despite the prevalence, maintaining a healthy lifestyle and achieving weight reduction remain critical for FLD prevention and no approved therapy exists for FLD. Thus, identifying safe and effective natural compounds for FLD management in animals is crucial for improving animal welfare and veterinary clinical outcomes (Tufarelli *et al.,* 2024; Jakimowicz *et al.,* 2025).

Autophagy, a conserved cellular process for degrading damaged organelles or lipid droplets, is central to hepatic lipid homeostasis (Filali-Mouncef *et al.,* 2022; Ren *et al.,* 2024). Multiple works have revealed impaired autophagy in the livers of FLD mouse models and patients, establishing an association between autophagy and FLD (Byun *et al.,* 2020; Wu *et al.,* 2020). In FLD, impaired autophagic flux leads to dysfunctional mitochondria, which exacerbate steatosis and inflammation in the liver (Chen and Lin, 2022). The recent study has reported that the autophagosome-lysosome fusion step is impaired in FLD (Cheng *et al.,* 2019; Lu *et al.,* 2023). Besides, autophagy dysfunction creates a vicious cycle of lipid accumulation and cellular damage, driving FLD progression in both humans and animals. Conversely, maintaining lysosomal acidification alleviates autophagic and metabolic disturbance in fat mice (Tanaka *et al.,* 2016). Therefore, a more comprehensive exploration of the mechanisms underlying autophagy in FLD is likely to lead to the discovery of novel therapeutic targets.

Chemokine receptor 1 (CCR1) is a G protein-coupled receptor encoded by the CCR1 gene and belongs to the chemokine receptor family. CCR1 features seven transmembrane domains, with its intracellular signaling motifs coupling to downstream cascades (Terasaki *et al.,* 2022). Earlier research has demonstrated that CCR1 induces immune cell chemotaxis, drives the inflammatory cytokine production, and enhances oxidative stress through upregulation of reactive oxygen species (ROS) production (Yan *et al.,* 2020; Barnes, 2022; Xu *et al.,* 2025). Emerging evidence has suggested there is a crosstalk between CCR1 signaling and autophagy. It has been demonstrated that the CCR1 antagonist BX471 promotes repair of damaged spinal cord tissues by enhancing autophagy in pathological conditions (Hasan *et al.,* 2025). Although the current findings suggest that CCR1 inhibits autophagy, its specific roles and underlying mechanisms in FLD remain to be further investigated.

Bruceine A (BA) is a quassinoid that derived from the traditional Chinese medicinal plant *Brucea javanica (L.) Merr.* (Nakao *et al.,* 2009) and has demonstrated potent anti-inflammation, antioxidant, and autophagy-modulation (Li *et al.,* 2022; Zhang *et al.,* 2023; Du *et al.* 2025). The recent study has revealed that BA suppressed the inflammatory mediator synthesis, and thereby significantly reversing hepatic fibrosis through NR2F2-modulated HMGB1 inflammatory signaling cascades in murine models (Sun *et al.,* 2025). Moreover, BA alleviated alcoholic liver disease via an FXR-dependent mechanism to inhibit AIM2 inflammasome activation (Li *et al.,* 2024b). Although the hepatoprotective effects of BA have been documented, which suggests its potential in FLD treatment (Li *et al.,* 2024b), its veterinary applications in preventing hepatic steatosis in domesticated species remain largely unexplored. Given the rising prevalence of FLD in animals, coupled with the limitations of existing interventions, there is an urgent need for safe and effective preventive strategies. Therefore, this study was planned to systematically investigate the hepatoprotective efficacy of BA against FLD and to elucidate the underlying mechanisms, thereby providing a fundamental basis for understanding its therapeutic potential in managing FLD.

MATERIALS AND METHODS

Ethical Statement: All authors indicate that all animal experiments comply with the ARRIVE guidelines and should be carried out in accordance with the UK Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments, or the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978). All animal care and laboratory procedures were strictly observed the Guide for Care and Use of Laboratory Animals and approved by Animal Ethics Committee of China Medical University (No. CMUKT2024170).

Animals and treatment schedule: Healthy male C57BL/6 mice (n=60) at the age of 6-8 week (w) were obtained from Beijing Huafukang Biotechnology Co., Ltd (Beijing, China). All mice were housed in standard laboratory environment. The FLD model was developed based on previously published work with appropriate modifications (Tong *et al.,* 2023). After adaption for one week, the mice were randomly divided into five groups: Normal control group (Con): Mice were fed with a normal diet (5% fat) for 16 weeks, and during the feeding period, the corresponding volume of normal saline was injected intraperitoneally every 4 days. Model group (FLD): Mice were subjected to a high-fat diet (HFD, 32% fat) for 16 weeks, and during the feeding period, the corresponding volume of normal saline was injected intraperitoneally every 4 days. BA low-dose group (FLD+BA-1mg/kg): Mice were subjected to HFD (32% fat) and during the feeding period, 1mg/kg BA was injected intraperitoneally every 4 days. BA medium-dose group (FLD+BA-2mg/kg): Mice were subjected to HFD (32% fat) and 2mg/kg BA was injected intraperitoneally every 4 days. BA high-dose group (FLD+BA-4mg/kg): Mice were subjected to HFD (32% fat) and 4mg/kg BA was injected intraperitoneally every 4 days. Body weights were monitored throughout the experiment. The behavioral indicators of mice (including spontaneous activity level, feeding frequency, and food intake amount) were recorded daily. At 16 weeks post-high-fat diet providing, the blood glucose was collected using commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Afterwards, the euthanized mouse was placed ventrally on a sterile dissection board, a 1.5-2cm midline abdominal incision was made to expose internal organs, and the liver was isolated by severing connecting tissues. Then, the liver were weighted and preserved for the following detection:

Hematoxylin-eosin (H&E) staining: The freshly collected liver tissues were fixed in 4% paraformaldehyde solution (Qiu *et al.,* 2023), dehydrated via graded ethanol, cleared with xylene, and then paraffin-embedded. Then, the samples were sectioned into slices at 5μm, which were dewaxed, rehydrated, stained with hematoxylin and counterstained with eosin (Solarbio, Beijing, China). Images were captured via Olympus light microscope (BX53).

Oil Red O staining: The abovementioned samples were stained with modified Oil Red O working solution (Solarbio, Beijing, China) at 37℃ for 20min (Jia *et al.,*

2019), differentiated with 60% isopropanol for 10s and counterstained with hematoxylin for 2min (Solarbio, Beijing, China). Images were captured via Olympus light microscope (BX53).

Oxidative stress indicator: The obtained liver samples were also used to detected the levels of superoxide dismutase (SOD), reactive oxygen species (ROS), malondialdehyde (MDA) and glutathione (GSH) using commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The concentrations or activities were calculated based on standard curves.

RNA-sequencing: Liver tissue samples were collected from rats in FLD group and BA high-dose group with total RNA extracted using the TRIzol reagent and RNA quantity was assessed via a NanoDrop ND-1000 spectrophotometer. High-throughput mRNA sequencing was implemented on an Illumina NovaSeq 6000 platform (Zhai *et al.* 2024).

Immunohistochemistry: The immunohistochemical procedure was consistent with the method described in previous study (Wang *et al.,* 2022b). The primary antibodies were list as follows: CCR1 (Abcam, ab140756) and Beclin1 (Abcam, ab210498).

RT-Qpcr: Total RNA was extracted from mouse liver tissues using TRIzol reagent (Invitrogen). The RNA samples were reverse transcribed into cDNA using Supermo III M-MLV reverse transcriptase (BioTeke Corporation, Beijing, China). For amplification, cDNA, CCR1-specific primers (Sangon Biotech, Shanghai, China), SYBR® Green I (Sigma, S9430), and 2×Power Taq PCR MasterMix (BioTeke Corporation, Beijing, China) were loaded onto the Exicycler 96 (Bioneer Corporation). All experimental procedures were performed according to previously published protocols with appropriate modifications (Zhai *et al.,* 2024). The relative expression level of CCR1 was calculated using the $2^{-\Delta\Delta Ct}$ method with each sample analyzed in triplicate. The primers were used for CCR1, 5'-TGGTGGGCAATGTCCTAGTGATTC-3', and 5'-ACAGCCAGGTTGAACAGGTAGATG-3'.

Transmission electron microscope analysis: The fresh liver tissues were processed and cut into small pieces. The livers were fixed in glutaraldehyde, post-fixed in osmium tetroxide, dehydrated with gradient ethanol, and embedded in epoxy resin. Ultrathin sections were prepared, stained with uranyl acetate and lead citrate, and observed under a transmission electron microscope to detect autophagosome (Wang *et al.,* 2022a).

Western blot analysis: Regarding the western blot detection of CCR1, L3II/I, P62, and Beclin1 expression in liver tissues, all the experimental procedures are based on the previously published work (Jiang *et al.,* 2021) . the primary antibodies were as follows: CCR1 (Abcam, ab233832), L3II/I (Abcam, ab192890), P62 (Abcam, ab109012) and Beclin1 (Abcam, ab302669).

Statistical analysis: Data analysis was performed using GraphPad Prism 9.0 and expressed as mean±SD. Statistical comparisons were made via one-way ANOVA with post-hoc analysis using Tukey's multiple comparison test. P value less than 0.05 was considered statistically significant.

RESULTS

BA improves general physiological abnormalities in HFD-fed mice: To explore the impact of BA (Fig. 1A) on FLD, mice were fed with HFD for 16w and treated with BA at different doses (Fig. 1B). HFD feeding typically leaded to hyperphagia and reduced locomotor activity in mice. Body weight and liver weight were monitored weekly. As shown in Fig. 1C, BA admimistration prevented weight gain in FLD mice with the high dose of BA showing a more obvious inhibitory effect ($P<0.05$). The BA-treated group showed a distinctly decrease in liver weight (Fig. 1D, $P<0.05$). Relative to the Control group, the FLD group showed increased TC, TG, and LDL levels alongside reduced HDL, with these changes could be reversed by BA treatment (Fig. 1E-H, $P<0.05$). Serum ALT and AST activity were determined to assess liver function after HFD fed. The FLD group showed higher ALT and AST levels compared to the Control group. In contrast, BA treatment

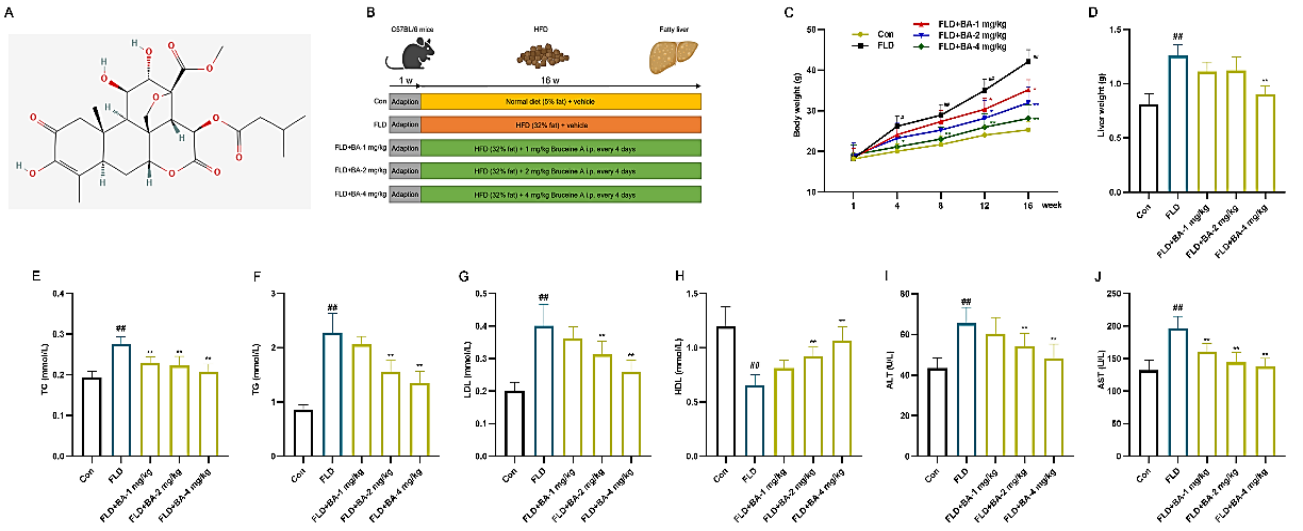

Fig. 1: BA alleviates FLD symptoms in mice fed with HFD. (A) Chemical structure of BA. (B) Experimental schedule. (C) and (D) presented body weight and liver weight changes over 16 w. (E)-(J) displayed serum levels of TC, TG, LDL, HDL, ALT, and AST. Data are represented as mean±SD (n=6) and are analyzed by one-way ANOVA. $^{\#}P<0.05$, $^{\#\#}P<0.01$ vs. Control group. $^{*}P<0.05$, $^{**}P<0.01$ vs. FLD group.

led to a decrease in ALT and AST levels (Fig. 1I-J, P<0.05), suggesting that BA treatment exerted a beneficial effect on FLD-fed mice.

BA inhibits hepatic lipid droplet accumulation in HFD-fed mice: Next, we observed no gross morphological differences among the groups, except for a slightly yellowish liver in the FLD group (Fig. 2A). Liver histopathological changes and lipid accumulation were assessed. As shown in Fig. 2B, the FLD group exhibited obvious pathological alterations, including disordered hepatic lobule arrangement, hepatocyte swelling and significant inflammatory cell infiltration. After treatment with BA, these pathological changes were mitigated by a dose-dependent manner. Fig. 2C demonstrated that there were small red lipid droplets in the liver cells of the Control group, while clearly distinct lipid accumulation in the liver of model mice. In the BA-treated groups, the number and size of lipid droplets in liver cells were reduced with BA treatment, indicating BA has the potential to improve liver histopathology and reduce lipid accumulation in FLD mice.

BA attenuates HFD-induced hepatic oxidative stress: To investigate the effect of BA on oxidative stress in FLD model mice, the liver was collected to determine the levels of SOD, GSH, MDA and ROS. As shown in Fig. 3A-B, SOD and GSH levels were significantly decreased (P<0.05), while the levels of MDA and ROS were

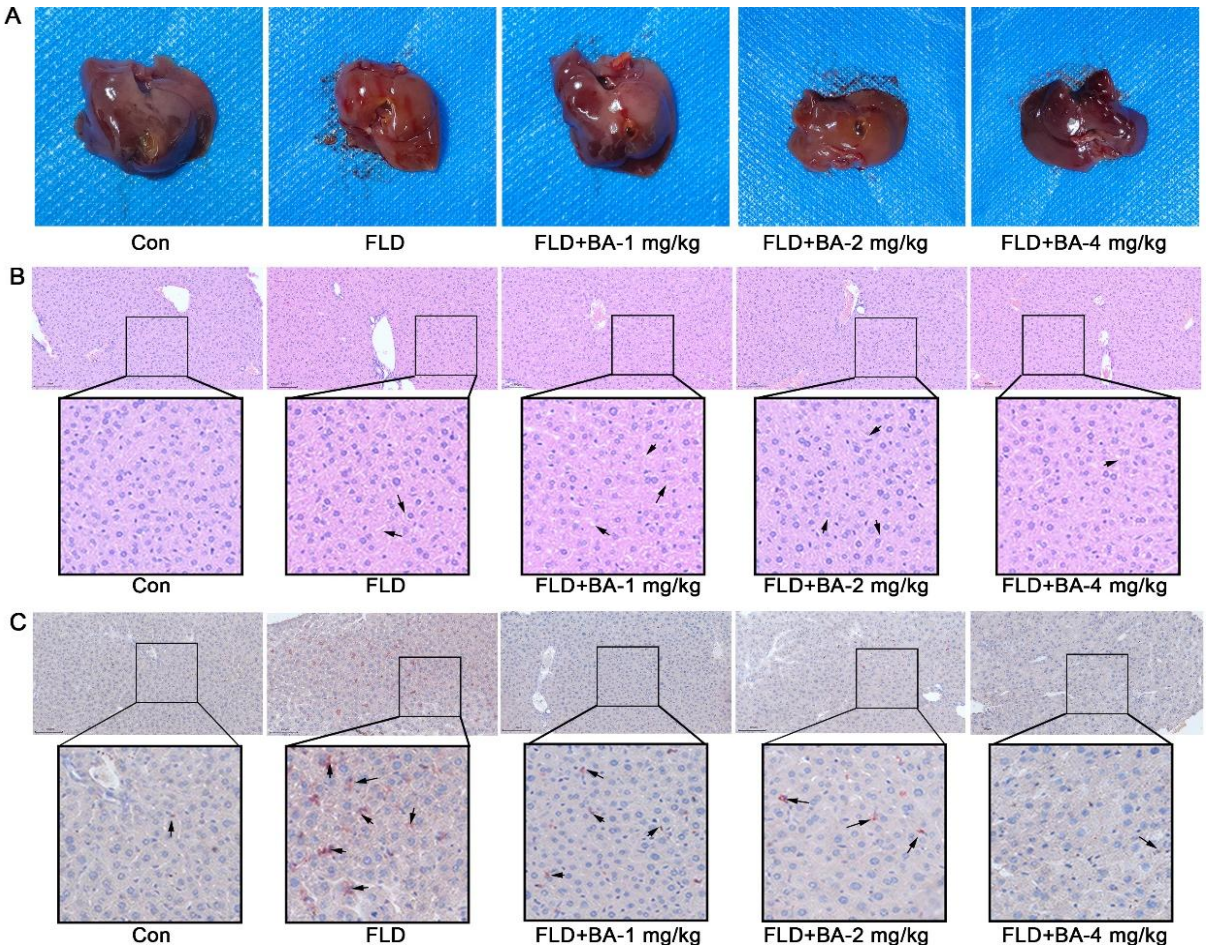

Fig. 2: BA inhibites lipid accumulation in FLD mice. Gross pictures (A) HE staining (B) and oil red O staining (C) of the liver tissues. The black arrows were used to indicate the corresponding pathological changes. (n=6).

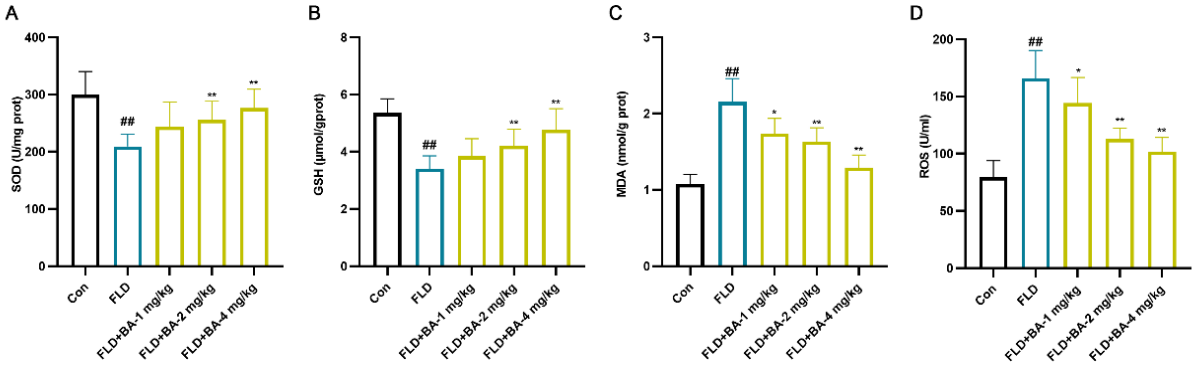

Fig. 3: BA abated oxidative stress injury in FLD mice. Liver SOD (A), GSH (B), MDA (C) and ROS (D) levels detected by commercial kit. Data are represented as mean±SD (n=6) and are analyzed by one-way ANOVA. ##P<0.01 vs. Control group. *P<0.05, **P<0.01 vs. FLD group.

obviously increased in the liver of FLD mice (Fig. 3C-D, P<0.05). After treatment with BA, these oxidative stress indicators of mice in BA-administrated groups were improved, with SOD and GSH levels increasing and MDA and ROS levels decreasing in a dose-dependent manner. These results suggested BA possessed the property to alleviate oxidative stress in FLD model mice.

BA reduces hepatic CCR1 protein expression in HFD-fed mice: The liver tissues from the BA-treated group exhibited up-regulation of a total of 145 differentially expressed genes (DEGs), while the down-regulation was observed in187 DEGs, including CCR1 (Fig. 4A). Gene Ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) database were used to conduct function enrichment analysis and explore potential pathways (Fig. 4B-C). CCR1 expression was relative low in the control mice as detected by RT-qPCR, western blot and immunohistochemistry method, while significantly upregulated in the FLD group, and gradually reduced with increasing BA concentrations (Fig. 4D-E, P<0.05). These findings indicated that BA exerted an inhibitory effect on the elevated CCR1 expression in the FLD liver tissues.

BA upregulates autophagy in the livers of FLD mice: Liver autophagosomes were observed in the FLD group and BA-treated group by transmission electron microscopy. As shown in Fig. 5A, the formation of

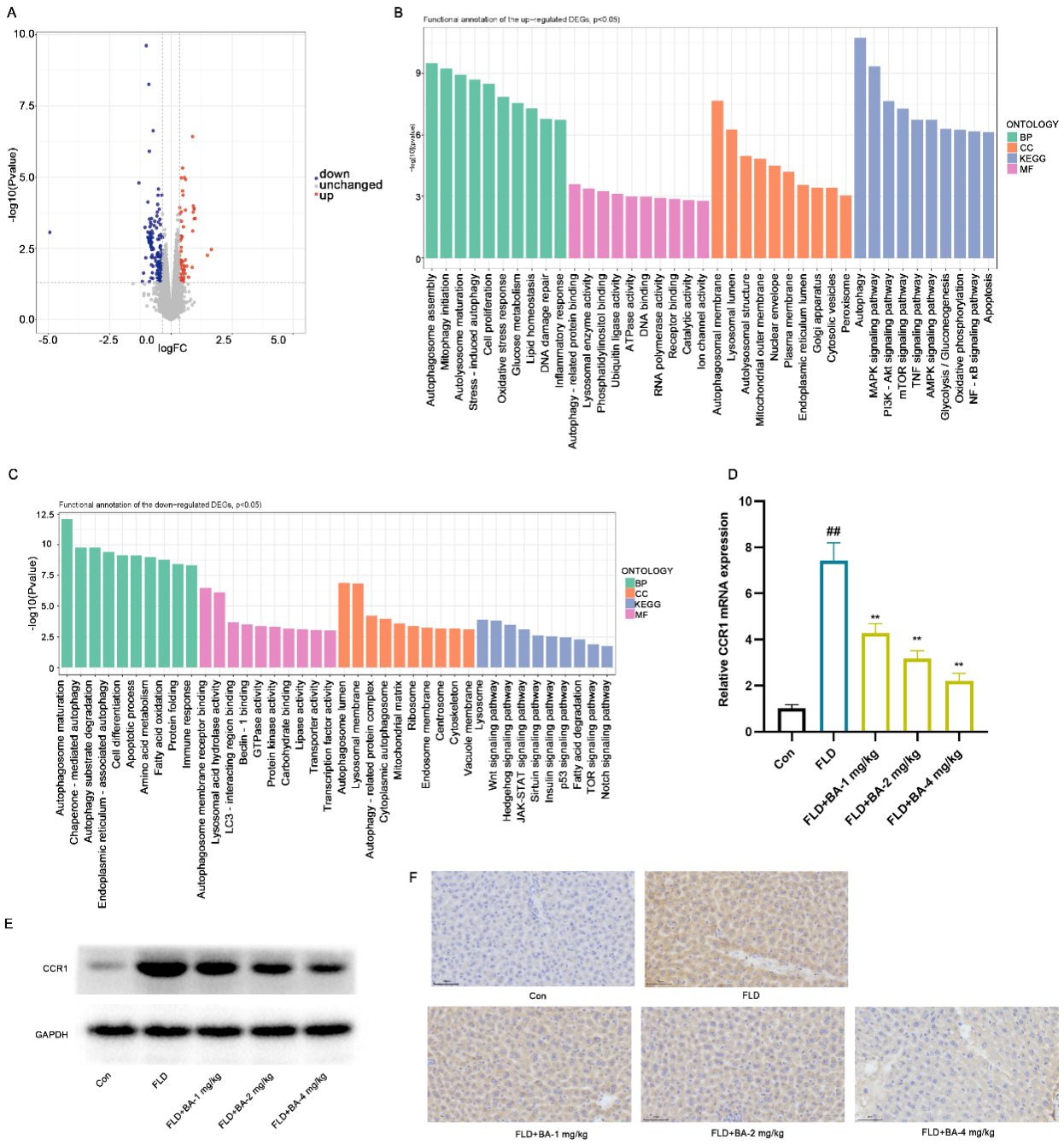

Fig. 4: BA attenuated CCR1 expression in FLD mice. (A) The volcano plot of the differentially expressed genes (DEGs) between FLD and BA-treated groups. (B) GO annotation and KEGG pathway analysis of the up-regulated DEGs. (C) GO annotation and KEGG pathway analysis of the down-regulated DEGs. (D) The mRNA expression of CCR1 in the liver tissues of FLD mice were detected by RT-qPCR. (E) The protein expression of CCR1 in the liver tissues FLD mice were detected by western blot. (F) Immunohistochemistry staining targeting CCR1 in the liver tissues FLD mice. Data are represented as mean±SD (n=6) and are analyzed by one-way ANOVA. $^{\#\#}$P<0.01 vs. Control group. *P<0.05, **P<0.01 vs. FLD group.

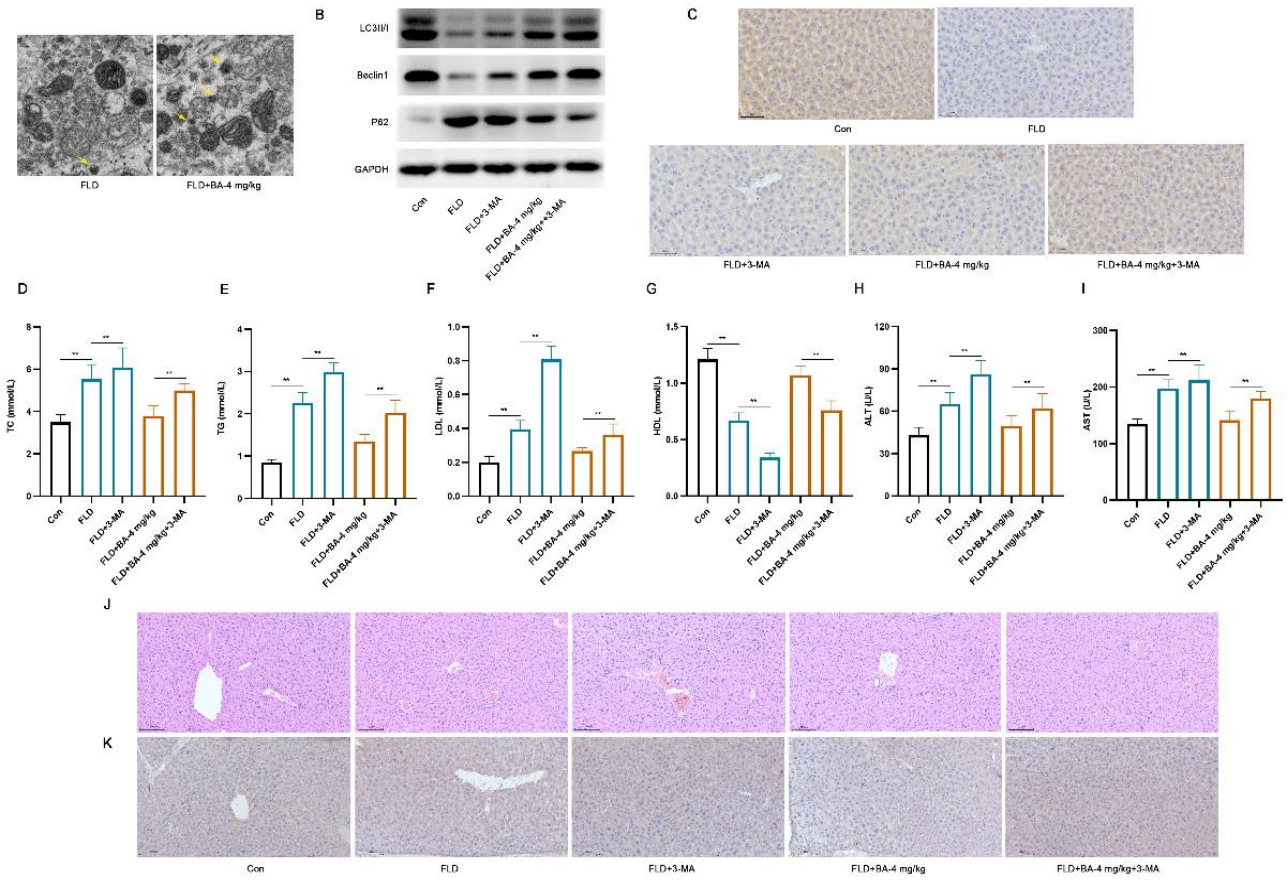

Fig. 5: BA alleviated FLD in mice possibly via promoting autophagy**.** (A) Transmission electron microscopy were used to shows liver autophagosome morphology. The yellow arrow: autophagosome. (B) The protein expression of LC3II/I, Beclin1 and P62 in the liver tissues FLD mice were detected by western blot. (C) Immunohistochemistry staining targeting Beclin1 in the liver tissues FLD mice. (D)-(I) displayed serum levels of TC, TG, LDL, HDL, ALT and AST. HE staining (J) and oil red O staining (K) of liver tissues. Data are represented as mean±SD (n=6) and are analyzed by one-way ANOVA. *P<0.05, **P<0.01.

autophagosomes increased after BA administration. Additionally, the levels LC3II/I and Beclin1 expression was relatively high in the control mice and decreased in the FLD group, while gradually increased with BA treatment (Fig. 5B). Besides, P62 expression showed the opposite trend (Fig. 5B). The following immunohistochemistry targeting Beclin1 confirmed the similar trends of western blot (Fig. 5C). To verify the role of autophagy in BA-mediated hepatoprotection, we used the autophagy inhibitor 3-MA. Specifically, BA-mediated protective effects, including maintaining normal serum lipid levels and liver function, could be reversed by the autophagy inhibitor 3-MA (Fig. 5D-K, P<0.05), which proved that BA exerted its effects by enhancing autophagy. Taken together, these findings indicated that BA alleviated FLD in mice possibly via autophagy promotion.

DISCUSSION

FLD can progress to life-threatening fibrosis or hepatocellular carcinoma, if left untreated. Diet-induced obesity and metabolic syndrome have contributed to the increasing prevalence of FLD in animals, which is linked to higher morbidity and mortality rates (Gerloff, 2000; Angeli *et al.,* 2019). Current therapeutic strategies for FLD mainly focus on lifestyle modifications, such as dietary restriction and exercise, but often fails to achieve sustained outcomes in clinical practice. Pharmacological interventions targeting metabolism, including insulin sensitizers and lipid-lowering agents, also show limited efficacy and safety in both humans and animals(Grummer, 2008; Wang *et al.,* 2024). Our study demonstrates that BA treatment effectively mitigates HFD-induced FLD in mice. Notably, this study is the first to reveal that BA exhibits a dose-dependent protective effect against weight gain and serum lipid abnormalities, accompanied by attenuated hepatic steatosis in mice exposed to long-term HFD Besides, the decreased oxidative damage could be found in the liver of mice after BA treatment. Mechanistically, BA exerts hepatoprotective effects through CCR1-mediated autophagic flux restoration. These findings support that the natural compound BA ameliorates induced FLD in mice by activating autophagy via CCR1 inhibition, thereby providing a novel perspective for refining treatment strategies against FLD.

It is well accepted that the long-term consumption of HFD inflicts severe and multifaceted damage to the liver, driving the progression of metabolic dysfunction and chronic liver disease through multiple mechanisms (Gao *et al.,* 2020; Lian *et al.,* 2020; Eng and Estall, 2021). Exercise and dietary intervention remain critical for FLD prevention and management with limited efficacy, it is necessary to investigate the underlying mechanism in order to develop new drugs (Huang *et al.,* 2021). To our knowledge, BA has been reported to exert therapeutic effects on diabetic nephropathy via inhibiting inflammatory response and oxidative stress injury (Li *et al.,* 2022). Accumulating studies have confirmed oxidative stress are important

factors that induce steatosis (González-Gallego *et al.,* 2011). Excessive lipid intake provides abundant substrates for oxidation, which promotes ROS production. Besides, excessively ROS directly oxidizes and damages DNA, proteins, and lipids within hepatocytes, which are closely associated with hepatocyte injury as well as the development of hepatic steatosis (Fujinaga *et al.,* 2011). Histopathological analyses, such as HE and oil Red O staining, confirmed BA could reduce hepatic lipid accumulation by a dose-dependent manner. Afterwards, BA treatment shows a beneficial effect in counteracting oxidative damage of the liver, evidenced by elevated SOD and GSH levels and reduced MDA and ROS production. Collectively, our findings demonstrate that BA mitigates HFD-induced hepatic steatosis and oxidative damage in a dose-dependent manner, suggesting its potential as a therapeutic agent for FLD in animals.

Next, to explore the potential targets of BA in alleviating HFD-induced liver damage, mRNA sequencing was performed. Among the differentially expressed mRNAs, CCR1 drew our attention. The recent study has expounded that CCR1 is implicated in liver inflammation and fibrosis by regulating immune cell recruitment and pro-inflammatory cytokine release (Li *et al.,* 2024a). Besides, the previous study also demonstrated that CCR1 depletion in mice challenged with a HFD-resulted in inhibited hepatic oxidative stress accompanied by a reduction in macrophage infiltration in the liver (Li *et al.,* 2024a). Similar to the results of above mentioned studies, our study showed that HFD feeding led to a dramatic upregulation of liver CCR1 at both the mRNA and protein levels, which was dose-dependently suppressed by BA, identifying CCR1 as a potential target of BA in FLD therapy. Previous studies have found that CCR1 promotes carcinogenesis in chronic lymphocytic leukemia and breast cancer by regulating AKT/mammalian target of rapamycin (mTOR) signaling (Shin *et al.,* 2017; van Attekum *et al.,* 2017). mTOR is a well-known key cellular sensor of nutrients that has been implicated in lipogenesis and hepatic steatosis in mice (Yan *et al.* 2022). It is well accepted that mTOR signaling pathway serves as a central regulator of autophagy. An increasing number of studies are focusing on the relationship between fatty liver disease and autophagy in dairy cows (Du *et al.,* 2018; Du *et al.,* 2024). Given that autophagy is regulated by mTOR through nutrient sensing, it is reasonable to hypothesize that CCR1 modulates autophagy, thereby establishing a connection between BA, oxidative stress, lipid metabolism, and autophagy regulation in the disease progression of FLD. In the present study, we observed that BA treatment led to an increase in autophagosome formation in the liver of FLD mice, along with an elevated in LC3II/I ratio, p62 degradation and Beclin1 expression. Meanwhile, 3-A (an autophagy inhibitor) was used to verify whether BA was involved in the progression of FLD through regulating autophagy. Fortunately, we found that treatment with 3-MA alone exacerbated these abnormalities, including further elevations in TC, TG, LDL, ALT, and AST, and a more significant reduction in HDL, indicating the aggravated lipid metabolism disorder and severe liver pathologic changes due to autophagy inhibition. Besides, the co-administration of BA and 3-MA could partially abolish the protective effect of BA, indicating that the hepatoprotective effects of BA could be partially attributed to its autophagy activation. Collectively, our findings suggest that BA alleviates HFD-induced liver damage by downregulating CCR1, thereby promoting autophagy, as evidenced by increased autophagosome formation.

Through the abovementioned experiments, our study conclusively demonstrated that BA exerted a significant ameliorative effect on induced FLD in mice. BA effectively alleviated HFD-induced weight gain and dyslipidemia in a dose-dependent manner, markedly mitigated hepatic steatosis, and reduced hepatic oxidative damage, thereby comprehensively improving the pathological progression of FLD. These findings not only provided new insights into the pathogenesis of FLD, but also opened a novel avenue for FLD treatment, demonstrating the great potential in veterinary medicine. Future research should focus on determining the optimal dosage, administration route, and treatment duration for BA to enhance its therapeutic outcomes while reducing potential toxicity.

Conclusions: In conclusion, our study demonstrates that BA ameliorates induced FLD in mice through promoting lipid clearance, attenuating oxidative stress, and activating autophagy via CCR1 inhibition. Notably, the protective effects of BA offer a novel therapeutic strategy for FLD in animals, thereby providing new insights for clinical translation.

Declaration of Competing Interest: The authors declare that he has no conflict of interest.

Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author's contribution: TY involved in study design and reviewed the final version, HHX and LSW wrote the manuscript and analyzed the data, LY completed experiments, LJP and WTF assisted with experiments and validation.

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