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Protective effects of baicalein treatment against the development of nonalcoholic steatohepatitis in mice induced by a methionine choline-deficient diet

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Conflict of Interest
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ABSTRACT

Purpose: Baicalein, a natural flavone found in herbs, exhibits diverse biological activities. Nonalcoholic steatohepatitis (NASH) is an irreversible condition often associated with a poor prognosis. This study aimed to evaluate the effects of baicalein on the development of NASH in mice.

Methods: Male C57BL/6J mice were randomly divided into four groups. Three groups were fed a methionine-choline-deficient (MCD) diet to induce NASH and were simultaneously treated with baicalein (at doses of 50 and 100 mg/kg) or vehicle only (sodium carboxymethylcellulose) through oral gavage for 4 weeks. The control group was fed a methionine-choline-sufficient (MCS) diet without the administration of baicalein.

Results: The baicalein treatment significantly reduced serum levels of alanine aminotransferase and aspartate aminotransferase, suggestive of reduced liver damage. Histological analysis revealed a marked decrease in nonalcoholic fatty liver activity scores induced by the MCD diet in the mice. Similarly, baicalein treatment at both doses significantly attenuated the degree of hepatic fibrosis, as examined by Sirius red staining, and hepatocellular death, as examined by the terminal deoxynucleotidyl transferase dUTP nick end labeling assay. Baicalein treatment attenuated MCD-diet-induced lipid peroxidation, as evidenced by lower levels of hepatic malondialdehyde and 4-hydroxynonenal, demonstrating a reduction in oxidative stress resulting from lipid peroxidation. Moreover, baicalein treatment suppressed hepatic protein levels of 12-lipoxygenase (12-Lox) induced by the MCD diet. In contrast, baicalein enhanced the activities of antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase. Additionally, baicalein treatment significantly reduced hepatic non-heme iron concentrations and hepatic ferritin protein levels in mice fed an MCD diet.

Conclusion: To summarize, baicalein treatment suppresses hepatic lipid peroxidation, 12-Lox expression, and iron accumulation, all of which are associated with the attenuation of NASH progression.

Keywords: baicalein; nonalcoholic steatohepatitis; iron; lipid peroxidation

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is a prevalent chronic liver condition worldwide [1]. Its severity ranges from simple steatosis to nonalcoholic steatohepatitis (NASH), which can progress to cirrhosis. Approximately 20% of the individuals with nonalcoholic steatosis develop NASH. While simple steatosis can be reversed with a relatively positive prognosis, NASH is an irreversible condition that is associated with poor outcomes such as fibrosis and liver failure [2]. Identifying effective approaches to prevent the development and progression of NAFLD, particularly NASH is of paramount importance.

Phytochemicals emerge as promising therapeutic options for preventing or treating liver damage, given their diverse biological activities and minimal adverse effects. Baicalein (5,6,7-trihydroxyflavone) is a natural compound found in the root of *Scutellaria baicalensis* and *Scutellaria lateriflora*. It possesses various pharmacological properties, including anti-inflammatory, anti-cancer, and antioxidant [3]. Existing evidence suggests that baicalein may have beneficial effects on liver function. Baicalein protects hepatocytes against tert-butylhydroperoxide-induced cytotoxicity *in vitro* [4]. In a screening study of a natural product library containing 1,130 chemicals, baicalein demonstrated the highest inhibitory effects against lipopolysaccharides/d-galactosamine-induced acute liver injury *in vitro* [5]. Baicalein exhibits hepatoprotective effects against carbon tetrachloride (CCl_4)-induced acute liver injury *in vivo* by stimulating hepatocyte regeneration [6]. Furthermore, baicalein reduced hepatic fat accumulation by activating AMP-activated protein kinase (AMPK) in mice fed with a high-fat diet [7]. High-fat diet induces hepatic steatosis without progression to steatohepatitis. However, it remains unclear whether baicalein has a protective effect against more advanced liver injuries such as NASH.

The methionine-choline-deficient (MCD) diet-induced NASH murine model is histologically similar to human NASH, and exhibits features such as hepatic steatosis, hepatocyte injury, inflammation, and fibrosis. In this study, we investigated the protective effects of baicalein against the development of NASH using an MCD diet-induced NASH murine model.

METHODS

Animals, diets, and experimental design

The 10-week-old male C57BL/6J mice (purchased from DBL Co., Eumseong, Korea) were randomly divided into four groups (n = 12/group). One control group (methionine-choline-sufficient, MCS) was fed a methionine and choline sufficient L-amino acid diet (A02082003BY; Research Diets, Inc., New Brunswick, NJ, USA), while the other three groups (MCD, MCD+B50, and MCD+B100) were fed a methionine and choline deficient L-amino acid diet (A02082002BR; Research Diets, Inc.). Baicalein (Tokyo Chemical Industry, Tokyo, Japan) was dissolved in a 0.5% sodium carboxymethyl cellulose (CMC-Na; Sigma-Aldrich, Saint Louis, MO, USA) solution. The MCD+B50 and MCD+B100 groups were supplemented with baicalein at a dose of 50 mg/kg body weight and 100 mg/kg body weight, respectively by oral gavage every day for four weeks. The MCD group was given only the vehicle (0.5% CMC-Na) by oral gavage. All mice had free access to drinking water and feed. At the end of the treatment, all mice were sacrificed under carbon dioxide anesthesia. Cardiac blood and liver tissue were collected and stored at −80°C until further analysis. All procedures were approved by the Kyung Hee University Institute of Animal Experimental Care and Use Committee (KHUIACUC; approval number: KHSASP-22-116).

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Histopathological evaluation

Liver tissues were fixed in 10% neutral buffered formalin and embedded in paraffin. Liver slices, 5 μm thick, were stained with hematoxylin & eosin (H&E) and Sirius red according to standard procedures. Histological examination was performed using the histological scoring system for NAFLD activity score (NAS) described by Kleiner et al. [8]. The NAS was calculated by averaging the observations from five fields of H&E-stained liver sections. Fibrosis was evaluated on Sirius red-stained liver sections by quantifying the percentage of red area compared to the total area using ImageJ software (National Institutes of Health, Bethesda, MD, USA). The averaging of 5 fields was determined. All stained liver sections were observed under an optical microscope (Olympus, Tokyo, Japan) at 200× magnification in a blinded manner.

Biochemical analyses

Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were determined using a commercial assay kit following the manufacturer's instructions (Asan Pharmaceutical Co., Ltd., Seoul, Korea). Liver antioxidant enzyme activities were assessed using commercial assay kits (Cayman Chemical, Ann Arbor, MI, USA). Hepatic malondialdehyde (MDA) concentrations were measured using a colorimetric assay with a commercial kit (Sigma-Aldrich).

Measurement of non-heme iron concentration

The concentration of non-heme iron in the liver was determined following the method described by Brain et al. [9]. Liver tissues were placed in an acid solution (3 M hydrochloric acid and 10% trichloroacetic acid solution) and incubated at 60°C for 20 hours. The resulting supernatant was then mixed with a chromogen reagent (0.1% bathophenanthroline sulphonate and 1% thioglycolic acid) and the optical densities were measured at 535 nm.

Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining

To assess the degree of hepatocyte cell death TUNEL staining was performed using a commercially available kit (Abcam, Cambridge, UK). TUNEL-positive cells were quantified under 200× magnification in 5 random fields, and the results were expressed as the mean area of TUNEL positive cells as a percentage of the total area using ImageJ software.

Western blots analysis

Liver tissues were homogenized in RIPA Buffer (Thermo Fisher Scientific, Rockford, IL, USA). The homogenates were then incubated at 4°C for 1 hour and centrifuged three times at 13,000 rpm for 15 minutes at 4°C to obtain supernatants. Protein quantification was performed using the BSA Protein Quantification Kit (Thermo Fisher Scientific). A 10–15 μg of protein was separated by 8–12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a polyvinylidene fluoride membrane. The membrane was then blocked in 5% skim milk with TBS-T (20 mM Tris, 150 mM NaCl, 0.1% Tween 20) for 1 hour at room temperature. All primary antibodies were incubated overnight at 4°C. Ferritin, 12-lipoxygenase (12-Lox), and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) antibodies were all purchased from Santa Cruz Biotechnology (Dallas, TX, USA), and 4-hydroxynonenal (4-HNE) antibody was purchased from R&D systems (Minneapolis, MN, USA). The membrane with the secondary antibody was incubated for 1.5 hours at room temperature. Detection was performed with ChemiScope (Clinx Science Instruments Co., Shanghai, China) using Clarity Western ECL Substrate (Bio-Rad, Hercules, CA, USA). The optical density of the band was quantified using ChemiScope Image analysis software (version 1.0; Clinx Science Instruments Co.).

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Statistical analysis

The data were analyzed using SAS 9.4 software and expressed as mean ± standard error of the mean. We performed a one-way analysis of variance and used Duncan's multiple range test for post hoc analysis. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Baicalein treatment protects against liver injury in MCD-induced NASH mice

To investigate the protective effects of baicalein against NASH development, we measured serum AST and ALT levels as indictors of liver injury. The MCD group, which received the MCD diet, showed a significant increase in serum AST and ALT activities compared to the control group, which received the MCS diet (**Fig. 1A and B**). However, the MCD diet-fed mice treated with baicalein at doses of 50 and 100 mg/kg (the MCD+B50 and MCD+B100 groups) exhibited a significant reduction in both serum AST and ALT activities. This indicates that baicalein effectively mitigated the increases induced by the MCD diet. Baicalein treatments at both doses did not significantly affect the mean body weights (data not shown).

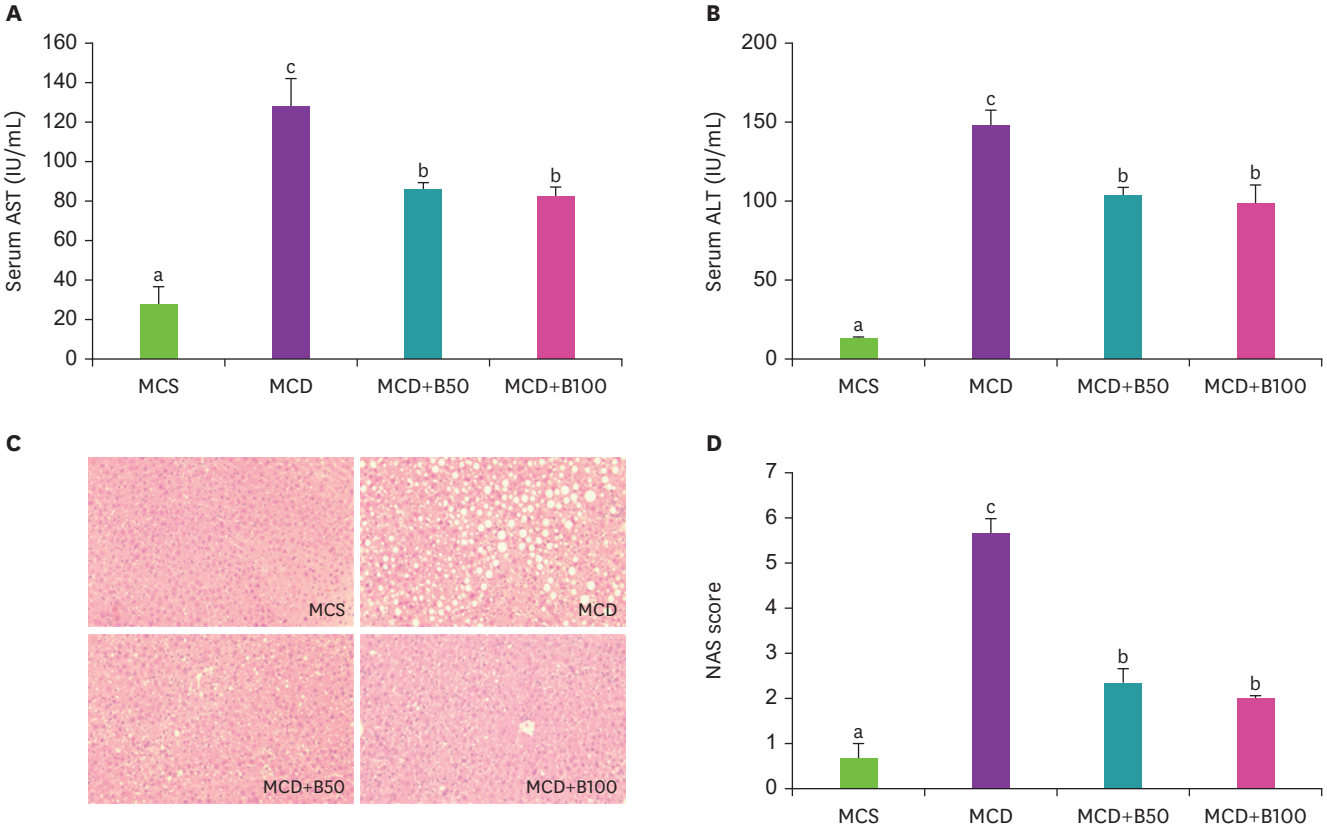

Fig. 1. Effects of baicalein treatment on liver injury in mice fed the MCD diet.
(A) Serum AST, (B) Serum ALT, (C) Representative images of liver sections stained with H&E (200× magnification). (D) Histological analysis using NAS. Data are presented as mean ± SEM (n = 12/group).
MCS, methionine-choline-sufficient; MCD, methionine-choline-deficient; MCD+B50, MCD with 50 mg/kg baicalein; MCD+B100, MCD with 100 mg/kg baicalein.
[a-c]Values with different superscripts are significantly different, as determined by ANOVA with Duncan's multiple range test.

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Histopathological analysis of liver sections stained with H&E revealed that the MCD fed group had significantly larger and more numerous fat droplets, increased lobular inflammation, and hepatocellular ballooning than the MCS group. The mean NAS were also significantly higher in the MCD group (**Fig. 1C**). However, baicalein treatment in the MCD+B50 and MCD+B100 groups remarkably alleviated these morphological changes compared to the MCD group treated with vehicle only. Additionally, both the MCD+B50 and MCD+B100 groups showed significant reductions (41% and 35.3%, respectively) in NAS when compared to the MCD group (**Fig. 1D**). Similar to findings in histopathological analysis, hepatic TG concentrations were significantly decreased in the MCD+50 and MCD+100 groups (62.5 ± 6.5 and 59.5 ± 3.7 mg/g tissue, respectively), compared to the MCD group (79.3 ± 5.1 mg/g tissue).

NASH-related fibrosis was assessed by examining Sirius red-stained liver sections. The MCD group showed a significant increase in red staining, indicating hepatic collagen deposition, compared with the control group fed with MCS. In contrast, baicalein treatment at doses of 50 and 100 mg/kg in the MCD-fed mice markedly reduced collagen accumulation by approximately five-fold compared to that in MCS-fed control mice (**Fig. 2A and B**).

We further investigated the effects of baicalein on hepatocellular death using the TUNEL assay. The MCD diet significantly increased hepatocyte apoptosis compared to the MCS diet. However, the area of TUNEL-positive cells in the livers was significantly reduced in the MCD+B50 and MCD+B100 groups, in comparison to that in the MCD group (**Fig. 2C and D**). This suggests that baicalein treatment effectively reduces hepatocellular death during progression to NASH.

Baicalein treatment reduces hepatic oxidative stress and lipid peroxidation in MCD-induced NASH mice

4-HNE and MDA are indicators of the oxidative stress resulting from lipid peroxidation. To assess the effect of baicalein treatment on hepatic oxidative stress, 4-HNE and MDA levels in the livers were measured. The results showed a significant increase in the protein levels of 4-HNE in the livers of mice fed the MCD diet compared to those in control mice fed the MCS diet (**Fig. 3A**). However, mice in the MCD+B50 and MCD+B100 groups exhibited significantly decreased hepatic 4-HNE levels compared to those in the MCD group. Similarly, hepatic MDA concentration was significantly increased in the MDA group by ten-fold compared to that in the MCS group (**Fig. 3B**). However, baicalein treatment at both doses significantly reduced the hepatic MDA concentrations induced by the MCD diet. The hepatic MDA concentrations in the MDA+B50 and MDA+B100 groups were 1.16 nmol/mg protein and 1.10 nmol/mg protein, respectively, compared to 2.02 nmol/mg protein in the MDA group ($p < 0.05$) (**Fig. 3B**).

In addition, we examined whether baicalein treatment affected the hepatic expression of 12-Lox by western blot analyses. 12-Lox enzyme belongs to lipoxygenase family that catalyzes the peroxidation of unsaturated fatty acids found in membrane lipids, such as arachidonate. We observed that the protein levels of 12-Lox in the MCD group were three times higher than to those in the MCS control group (**Fig. 3C**). However, baicalein treatment at both doses suppressed the hepatic expression of 12-Lox induced by MCD diet.

Baicalein treatment enhances antioxidant enzyme activity and reduces hepatic iron accumulation in MCD-induced NASH mice

To study the antioxidant effects of baicalein in preventing NASH progression, we measured the activities of antioxidant enzymes in liver homogenates including superoxide dismutase, catalase, and glutathione peroxidase (**Fig. 4**). Superoxide dismutase activity was

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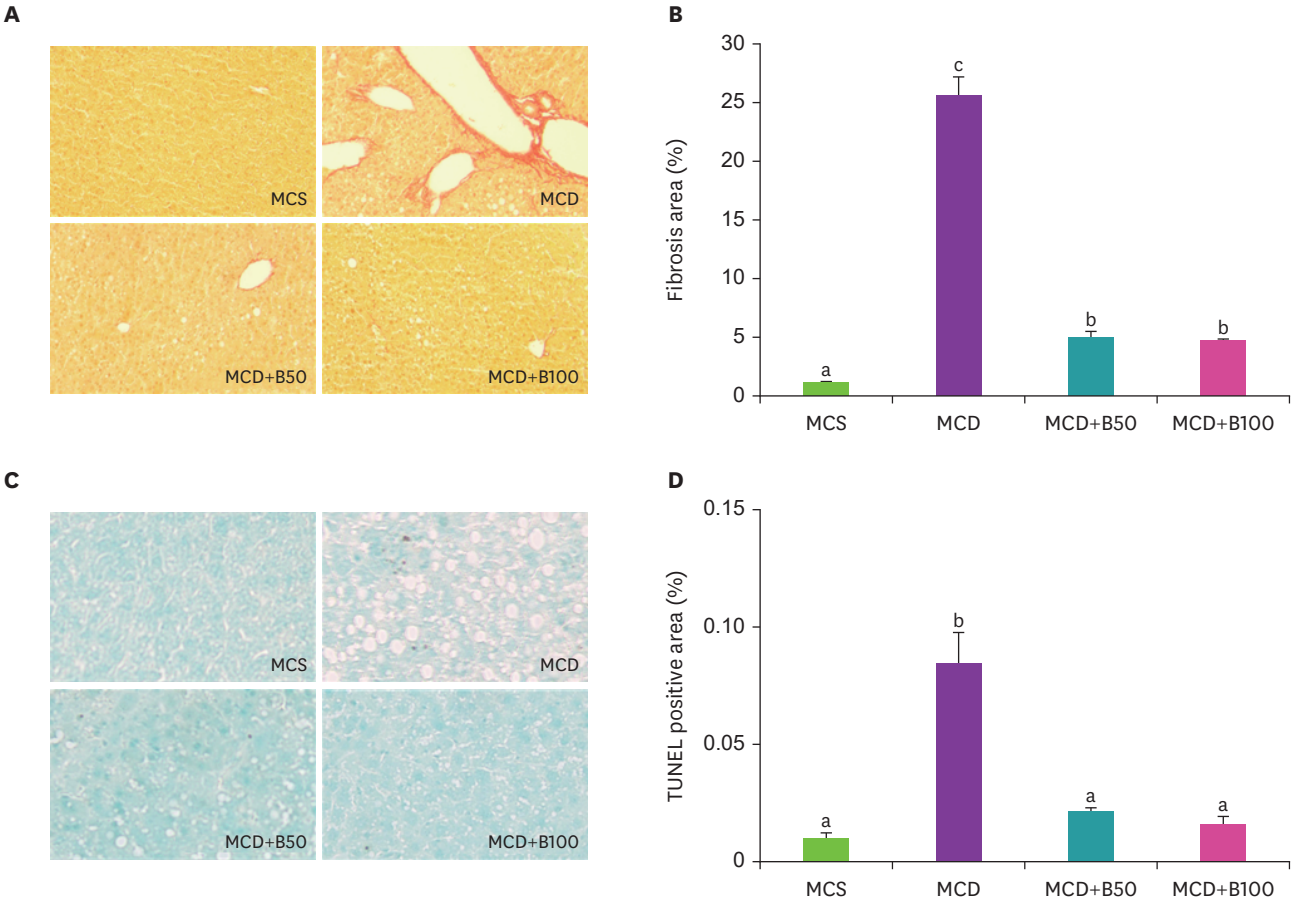

Fig. 2. Effects of baicalein treatment on hepatic fibrosis and hepatocyte cell death in mice fed the MCD diet.
(A) Representative images of Sirius red staining of liver sections, (B) Quantitative analysis of Sirius red staining positive area expressed as a percentage of total area, (C) Representative images of TUNEL staining. (D) The quantification of TUNEL positive area as percentage of total area. Data are presented as mean ± SEM (n = 12/group).
MCS, methionine-choline-sufficient; MCD, methionine-choline-deficient; MCD+B50, MCD with 50 mg/kg baicalein; MCD+B100, MCD with 100 mg/kg baicalein.
[a-c]Values with different superscripts are significantly different, as determined by ANOVA with Duncan's multiple range test.

significantly lower in the MCD group than in the MCS group. Baicalein treatment at both doses significantly increased hepatic superoxide dismutase activity compared to the MCD group treated with vehicle only (**Fig. 4A**). Catalase activity did not differ between the MCS and the MCD groups. However, baicalein treatment at a dose of 100 mg/kg significantly increased catalase activity compared to the MCS and the MCD groups (**Fig. 4B**). There was no difference in hepatic glutathione peroxidase activity between the MCS and MCD groups. However, baicalein treatment increased glutathione peroxidase activity in a dose-dependent manner. Glutathione peroxidase activity in the MCD+B50 and MCD+B100 groups was 2.7- and 8.9-fold higher compared to the MCD group (**Fig. 4C**).

The hepatic non-heme iron concentration was markedly higher in the MCD group than in the MCS group (**Fig. 5A**). Hepatic non-heme iron concentrations in the MCD+B100 group were significantly lower compared to the MCD group. Likewise, the levels of ferritin, an iron storage protein, were significantly elevated in the MCD group compared to those in the MCS group. Mice fed the MCD diet with 100 mg/kg baicalein showed a significant decrease in liver ferritin protein levels compared to mice fed the MCD diet alone (**Fig. 5B**).

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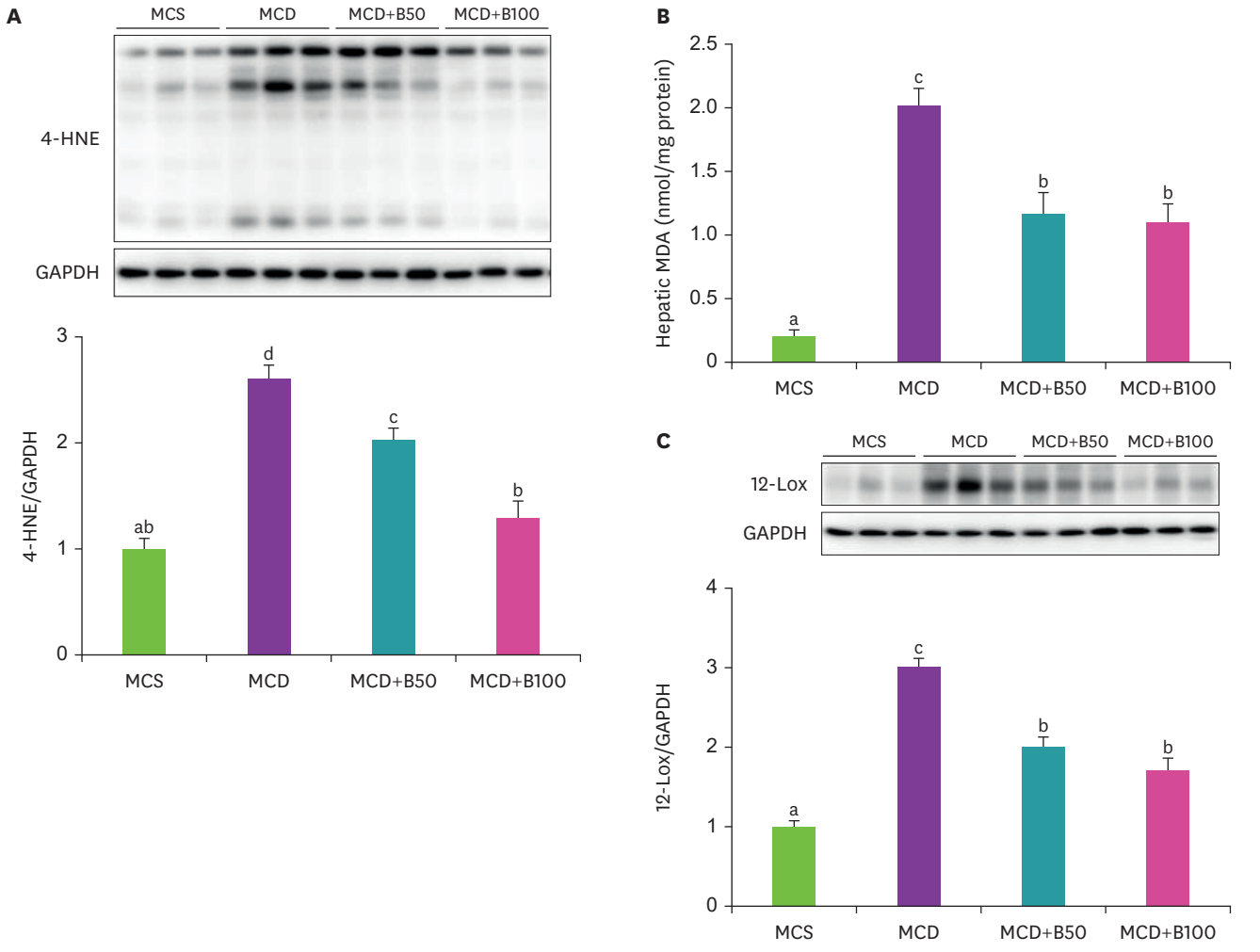

Fig. 3. Effects of baicalein treatment on hepatic lipid peroxidation and 12-Lox expression in mice fed the MCD diet.
(A) The levels of 4-HNE in the liver tissues detected by Western blot analyses. (Upper) Representative blots of 4-HNE (76 kDa) and GAPDH (36 kDa), (Lower) Quantitative analyses. (B) Hepatic MDA formation. (C) The protein levels of 12-Lox in the liver tissues detected by Western blot analyses. (Upper) Representative blots of 12-Lox (70 kDa) and GAPDH (36 kDa), (Lower) Quantitative analyses. Data are presented as mean ± SEM (n = 12/group).
MCS, methionine-choline-sufficient; MCD, methionine-choline-deficient; MCD+B50, MCD with 50 mg/kg baicalein; MCD+B100, MCD with 100 mg/kg baicalein.
[a-d]Values with different superscripts are significantly different, as determined by ANOVA with Duncan's multiple range test.

DISCUSSION

In the present study, baicalein effectively reduced the progression of NASH. Baicalein treatment significantly inhibited the development of hepatic steatosis, histopathological changes, hepatocellular death, and fibrosis induced by the MCD diet. There was a significant decrease in hepatic lipid peroxidation, accompanied by an increase in antioxidant activity and a decrease in hepatic 12-Lox expression and hepatic iron accumulation.

The progression of NASH is influenced by a complex interplay of factors including genetics, environment, insulin resistance, and oxidative stress [10-12]. The "two-hit" theory suggests that liver damage occurs as a result of the first hit caused by insulin resistance and the second hit caused by various oxidative stresses [13]. However, multiple molecular pathways are involved in the development of NASH. Recent studies have shown that lipotoxicity

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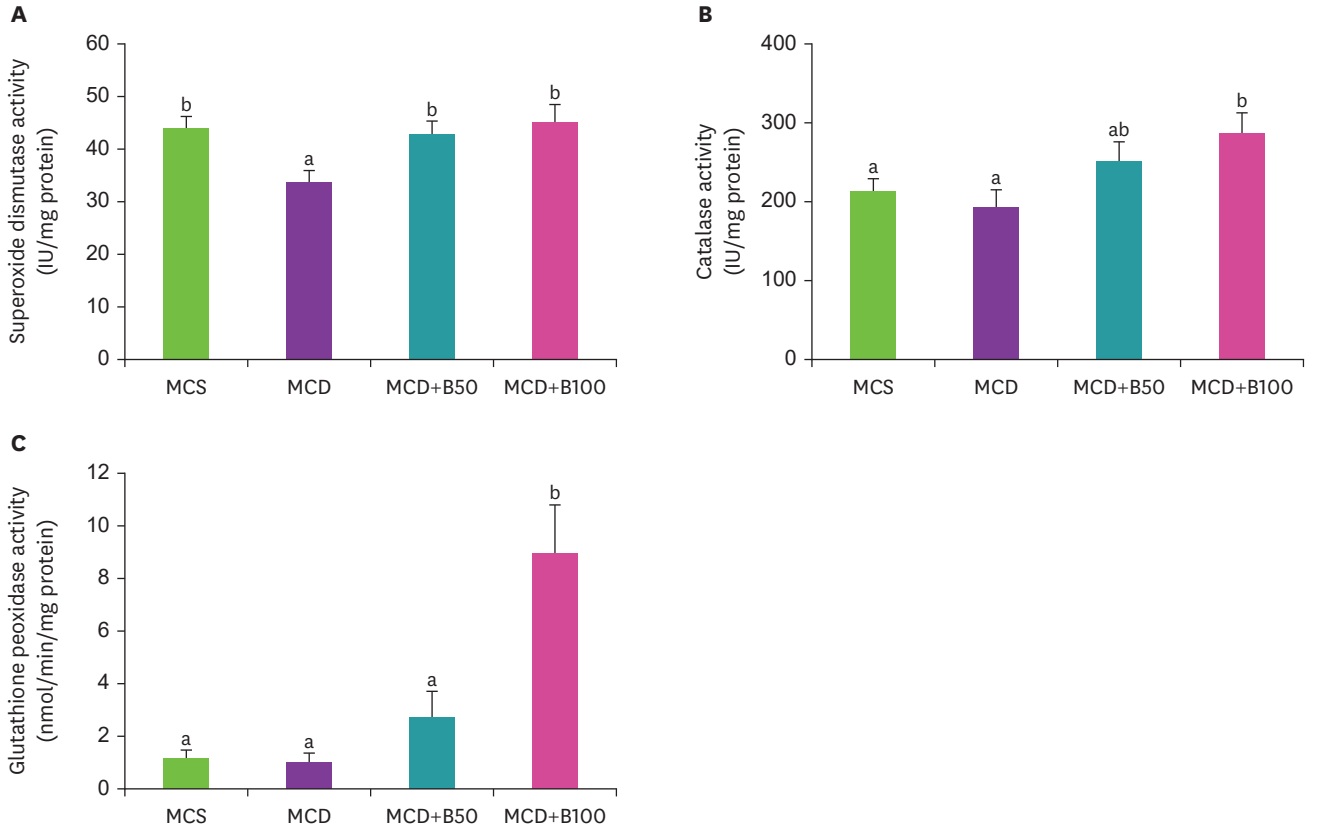

Fig. 4. Effects of baicalein treatment on hepatic activities of antioxidant enzymes in mice fed the MCD diet.
(A) Superoxide dismutase activity, (B) Catalase activity, and (C) Glutathione peroxidase activity. Data are presented as mean ± SEM (n = 12/group).
MCS, methionine-choline-sufficient; MCD, methionine-choline-deficient; MCD+B50, MCD with 50 mg/kg baicalein; MCD+B100, MCD with 100 mg/kg baicalein.
[a,b]Values with different superscripts are significantly different, as determined by ANOVA with Duncan's multiple range test.

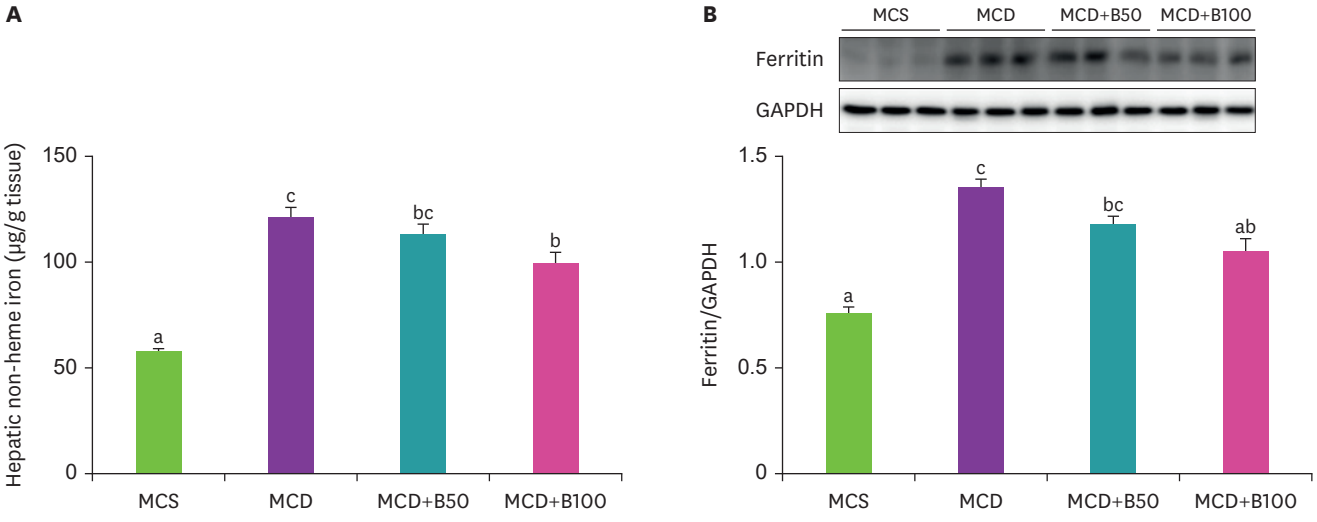

Fig. 5. Effects of baicalein treatment on hepatic iron accumulation in mice fed the MCD diet.
(A) Hepatic non-heme iron concentration, (B) The protein levels of ferritin (20 kDa) in the liver tissues detected by Western blot analyses. Data are presented as mean ± SEM (n = 12/group).
MCS, methionine-choline-sufficient; MCD, methionine-choline-deficient; MCD+B50, MCD with 50 mg/kg baicalein; MCD+B100, MCD with 100 mg/kg baicalein.
[a-c]Values with different superscripts are significantly different, as determined by ANOVA with Duncan's multiple range test.

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leads to hepatocellular death, triggering inflammation and fibrosis, which contribute to the progression of NASH [14]. Patients with NASH exhibit a significant increase in the number of TUNEL-positive hepatocytes in the liver [15]. Hepatic iron accumulation has also been implicated in NASH pathology [16]. Strategies such as phlebotomy or the use of iron chelators such as deferoxamine have been employed to prevent or alleviate NASH progression, but they often have adverse effects.

Flavonoids, natural compounds found in plants, have been extensively studied in NASH models. The beneficial effects of flavonoids on NASH are primarily attributable to their antioxidant properties [17]. Excess fatty acids in nonalcoholic steatosis lead to an increase in reactive oxygen species (ROS) production, which further promotes the progression from simple steatosis to NASH [18]. Flavonoids possess structural properties that enable them to effectively scavenge ROS [19]. Moreover, both *in vivo* and *in vitro* studies have shown that flavonoids exert positive effects on the progression of NASH by reducing inflammation and modulating lipid metabolism [20-22].

Baicalein, a flavonoid, has been reported to effectively alleviate liver diseases. For example, baicalein reduced hepatic fat accumulation and improved lipid profile abnormalities in mice with high-fat diet-induced NAFLD by activating AMPK and suppressing SREBP1 cleavage [7]. In our study, baicalein effectively reduced hepatic steatosis in MCD-fed mice, based on histopathological analyses. Baicalein also inhibits hepatocellular death, inflammation, and fibrosis, reflecting the range of changes observed in NASH-related hepatic pathology. Although NAFLD is often associated with metabolic syndromes such as obesity, hypertension, type 2 diabetes, and dyslipidemia due to insulin resistance, it can also occur in non-obese individuals. In fact, approximately 9–18% of patients with NAFLD are not obese. Unlike a high-fat diet, an MCD diet induces hepatic steatosis in the absence of obesity. Our findings suggest that baicalein may mitigate NASH through mechanisms independent of the previously reported modulation of lipid metabolism.

Oxidative stress is one of the mechanisms that drives the initiation and progression of NASH [23]. Antioxidant enzymes are essential for counteracting harmful free radicals and mitigating oxidative stress. Reduced catalase and superoxide dismutase activities are associated with the severity of liver disease [24]. Moreover, decreased glutathione peroxidase activity was associated with elevated hepatotoxicity induced by ferric nitrilotriacetate in mice [25]. Baicalein has been shown to have antioxidant properties in several disease models [26,27]. In our study, we observed that baicalein supplementation enhanced the enzymatic activities of glutathione peroxidase, catalase, and superoxide dismutase in the livers of mice fed the MCD diet. Furthermore, baicalein treatment reduced the levels of 4-HNE and MDA, which are indicators of lipid peroxidation. Considering glutathione peroxidase activity inhibits lipid peroxidation in membranes, these findings suggest that the antioxidant properties of baicalein contribute to the amelioration of NASH progression.

Our study found that hepatic non-heme iron concentrations were significantly higher in the MCD group compared to the MCS control group, despite both groups having the same dietary iron contents. Consistently, the hepatic levels of ferritin, a marker of cellular iron concentration, were also higher in the MCD group. A study conducted by Palladini et al. [28] found that iron levels in both the serum and liver increased as the disease progressed from steatosis to steatohepatitis after an 8-week MCD diet. This indicates a close association between iron status and disease progression. Another study demonstrated that iron overload

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in genetically obese mice exacerbated NASH progression by increasing hepatic oxidative stress, immune cell activation, and hepatocellular ballooning injury [29]. Patients with NAFLD also exhibit increased liver iron deposition associated with advanced fibrosis. Excessive iron can act as a pro-oxidant, generating reactive oxygen species and causing oxidative stress that damages liver cells. Recent studies have revealed the involvement of ferroptosis, a type of iron-dependent, non-apoptotic cell death, in NASH progression [30,31]. Ferroptosis induces cell death by directly destroying the cell membrane through excessive lipid peroxidation and the generation of lipid reactive oxygen species. In our study, the livers of mice fed the MCD diet displayed significantly higher levels of 4-HNE and MDA, indicating increased lipid peroxidation and elevated iron content compared to control mice. These findings suggest that ferroptosis plays a role in the progression of NASH.

After administering baicalein at a dose of 100 mg/kg, we observed a significant reduction in hepatic non-heme iron concentration and hepatic ferritin levels, compared to the vehicle-treated MCD group. Previous reports have indicated the iron-binding capacity of baicalein under physiologically relevant conditions, and it has been shown to effectively inhibit iron-promoted Fenton chemistry through a combination of chelation and radical-scavenging mechanisms [32]. Additionally, baicalein has been identified as the most potent natural product inhibitor of ferroptosis through screening of a natural product library. In an *in vitro* study using pancreatic cancer cells, baicalein suppressed iron accumulation and lipid peroxidation in erastin-induced ferroptosis. Consistent with these findings, baicalein alleviates brain injury by inhibiting ferroptosis [33]. Lipid peroxidation and abnormal iron metabolism are crucial contributors to ferroptosis. Our study demonstrated a significant decrease in hepatic 4-HNE and MDA levels as well as hepatic iron concentrations with baicalein treatment, suggesting a potential mechanism for the alleviation of NASH progression.

Baicalein has been reported to inhibit 12-Lox, an iron-containing dioxygenase that mediates the generation of membrane lipid hydroperoxides involved in the progression of ferroptosis. Our findings show that the expression of 12-Lox was significantly higher in the liver tissue of MCD-fed mice than those fed with the control MCS diet. Consistent with our finding, Li et al. [31] demonstrated the elevated metabolism of arachidonate, the major substrate of 12-Lox, during MCD feeding and showed that hepatic ferroptosis plays a critical role in NASH progression. Interestingly, our study showed a dose-dependent decrease in the hepatic protein levels of 12-Lox following baicalein treatment in the MCD diet-fed mice. Similarly, baicalein inhibits ischemia-reperfusion-induced injury in rats by inhibiting 12-Lox activity [34]. These findings are also supported by a previous study showing that baicalein treatment significantly inhibits hepatic 12-Lox expression in mice with acute liver injury induced by CCl_4 intoxication [35]. Additionally, this study reported that baicalein treatment protected against the CCl_4-induced cytotoxicity in HepG2 cells and silencing the *ALOX12* gene encoding 12-Lox partially abolished the protective effect of baicalein. Previous studies have also demonstrated a close correlation between hepatic 12-Lox expression and NASH severity, and treatment with ML355, a specific inhibitor of 12-Lox, blocks NASH progression in a lipotoxicty-derived NASH murine model [36]. Collectively, our findings suggest that suppression of hepatic 12-Lox expression by baicalein plays a role in mitigating NASH progression. Further investigation is needed to determine whether the inhibitory effect of baicalein on hepatic12-Lox expression affects the extent of ferroptosis in liver tissue during NASH progression.

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SUMMARY

This study provides evidence that treatment with baicalein can effectively reduce hepatic steatosis, hepatocyte death, histological changes, and fibrosis in a murine model of NASH induced by a MCD diet. The hepatoprotective effects of baicalein are attributed to its antioxidant properties and ability to inhibit hepatic iron overload, lipid peroxidation, and hepatic 12-Lox induction. These findings strongly suggest that baicalein has the potential to be used as a dietary supplement for the prevention and treatment of NASH.

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